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CERTAIN ASPECTS OF THE VIRUS DISEASES

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INTRODUCTION

Studies of the rosette disease and a foliage mottling of wheat (26, 28) have shown that these disorders behave in many ways like certain of the mosaic diseases. The association of cell inclusions (28) with the diseased wheat plants has naturally led to an inquiry into the literature dealing with cellular pathology, and as the investigations of rosette and cell inclusions have progressed it has become necessary to obtain a knowledge of some of the more general phases of the virus problem as it applies to both plants and animals.[2]

Although it is fully recognized that the virus diseases of man and other animals are independent of those occurring in plants, it is evident that certain aspects of the virus problem are of general biological significance. This appears to be especially true of the viruses themselves and of the pathology of the host cells. The students of animal pathology have spent more time in the study of the viruses and diseased cells than have plant pathologists, and some of the results obtained and theories advanced by the former have suggested the possibility that these may apply directly or indirectly to the virus diseases of plants. The writer intends to consider some of these phases briefly. The animal virus diseases have already been summarized by Wolbach (44), Arkwright (3), Simon (38), and others.

VIRUS DISEASES OF ANIMALS

Although the mosaic of tobacco (4) was the first disease demonstrated to be caused by a filterable virus, it is not surprising that the virus causal

[1] The substance of this report was submitted to the Office of Cereal Investigations and the chief of the Bureau of Plant Industry on March 10, 1924.

[2] Through the kindness of Dr. R. W. Hegner, Dr. C. E. Simon, and others at the School of Hygiene and Public Health, Johns Hopkins University, the writer was enabled to study their splendid collection of cell inclusions associated with certain virus diseases of man and the lower animals and to get in touch with many of the virus-disease problems.

agents have received greater attention in their relation to man and the lower animals than to plants. The virus diseases of animals have been studied from many angles, and marked progress has been made, especially in connection with the control of many of them.

At the present time there appear to be some fifty or more of these diseases, and in most of them their viruses pass some of the standard types of earthen or porcelain filters. Numerous data have been obtained which show that many of these viruses differ greatly in their ability to pass filters of different porosities. These differences have been attributed largely to supposed differences between the sizes of the infective particles. However, it has been pointed out by several workers that other factors, such as the possible plasticity of the particles, the concentration of the virus, and the nature of the fluid containing the virus, tend to influence filterability.

Many of the animal viruses have also been studied to determine the influence of various chemicals, disinfectants, temperatures, and other factors on their infective properties. Most of the animal viruses are inactivated at temperatures between 50° and 60° C. Low temperatures, freezing and thawing, and desiccation have little detrimental effect on many of them. A good many of the viruses are surprisingly resistant to glycerin, alcohol, and disinfectants.

In addition to the study of the viruses themselves, considerable attention has been devoted to the diseased cells of the various hosts. In many cases certain cells of the affected animals show fairly definite inclusions, which have been studied by many investigators and are interpreted in several ways. These interesting bodies are associated with 20 or more of the virus diseases, and it is thought that they may occur in association with a still greater number of these maladies. In some cases there is a question as to the constancy of the occurrence of inclusions with a given disease; but in many instances they seem to be rather definitely associated, even to the extent that they may be used as a basis for diagnosis, as in the case of the Negri bodies of rabies. Many workers believe that the presence of inclusions in the cells of an animal affected by an unknown malady gives good reason for studying the disease according to virus methods. On the other hand, there are those who scout this idea.

Several distinct types and forms of cell inclusions have been found associated with these diseases, and Lipschütz (24) has classified them on the basis of their relation to the contents of the cell, dividing them into three groups as follows:

(1) Cytooikon group: Inclusions present in the cytoplasm.

(2) Karyooikon group: Inclusions present in the nucleus.

(3) Cytokaryooikon group: Inclusions present in the cytoplasm and the nucleus.

The exact nature of cell inclusions has been a much debated question. Calkins (8) conducted morphological studies on the Guarnieri bodies associated with smallpox (variola) and considered them protozoa and the parasites causing the disease. Negri (31), who discovered and studied the cell inclusions associated with rabies, also concluded that they are protozoan parasites and the cause of the disease. These workers observed minute granules in association with the larger inclusions and considered these the regenerative forms (gemmules of Calkins) produced by the larger body, or "protozoan."

Halberstaedter and Prowazek (16) studied the morphology of the cell inclusions and minute granules associated with the trachoma disease of the human eye and concluded that the inclusions themselves are not organisms at all but merely products secreted by the nuclei of the diseased cells. They[3] believed that the minute granules are true organisms and the etiological agents, that these "organisms" stimulate the cell nucleus to extrude nucleolar substance ("plastin") into the cytoplasm, and that this material forms the inclusions which serve as "mantles" for the minute "organisms." They called these supposed organisms "Chalamydozoa," on account of this apparent mantle, and applied their theory to the cell inclusions and microscopic granules associated with other virus diseases of animals. Lipschütz (23) named the minute granules or supposed organisms "Strongyloplasmen." He and other workers believe that these granules pass through bacteria-proof filters.

Attempts to cultivate the animal viruses *in vitro* have been numerous. In some cases positive results appear conclusive, and at the present time Arkwright (3) considers that four "viruses" (of pleuropneumonia of cattle, avian diphtheria, influenza, and poliomyelitis) have been cultured successfully. Noguchi (32, 33) applied culture methods to the study of the virus of rabies and that of trachoma. In his work on the rabies virus he states that the "parasite" grew in culture, and his report indicates that he obtained stages in the culture solution which were similar to the Negri bodies and the minute granules found in certain cells of rabid animals. He claims to have obtained the typical symptoms of rabies in animals which he inoculated with culture material containing these "granular, pleomorphic or nucleated bodies." The photographic evidence which Noguchi produced on this point indicates that his culture solutions did contain structures very similar to Negri bodies. In the light of this behavior Noguchi considered that the Negri-like or "nucleated bodies" are independent organisms which exhibit the appearance of protozoa. He does not mention any controls for

[3] It is generally accepted that von Prowazek developed this and the Chlamydozoan theory.

these experiments. Noguchi (33) also claims to have cultured an organism from trachoma. He reports that these organisms showed morphological features similar to typical trachoma inclusions, and his photographic evidence seems to bear out his claim. However, these cultures did not produce the disease when used in inoculation experiments.

Moon (30) attempted to cultivate *in vitro* the virus of rabies, and although he makes no definite claims he considers that he obtained an increase in the amount of virus. He reports that bodies similar to Negri bodies developed in some of his cultures. He points out, however, that in certain cases degenerated brain tissue in his culture tubes might cause cell nuclei to be confused with Negri bodies. Poor and Steinhardt (36) also attempted to cultivate the virus of rabies but report negative results. The methods employed by these workers differed from those of Noguchi and Moon. Steinhardt and her co-workers (40, 41) report the culture of the virus of cowpox (vaccinia) in tissue cultures, and although they claim to have produced the disease from the third subculture from the original planting they did not obtain cell inclusions. They did find minute granules which were similar to those in diseased cells, but these were in their controls also. Several other workers have attempted to cultivate the viruses of rabies, trachoma, and vaccinia but without success. At the present time many investigators consider that these as well as many other viruses have not yet been successfully cultured *in vitro*.

The theories which have been advanced concerning cell inclusions and the small granules associated with certain virus diseases have met with rather wide acceptance for a time among certain groups of workers. In general, it appears that the ideas of Prowazek and Lipschütz are now entertained by a great many investigators. Others, however, are holding open minds as to the nature of cell inclusions and the microscopic granules until more experimental data are available.

VIRUS DISEASES OF PLANTS

The main features of the virus diseases of plants have already been summarized by Dickson (9), Butler (7), and others; therefore it is not necessary to go into the general subject fully at this time. There are several distinct types of infectious diseases of unknown cause which are characterized by chlorosis and several other types of symptoms, such as foliage distortions, blight, excessive green coloration, arrested growth, proliferation, internal lesions, and galls; and some of these diseases may manifest nearly all of these symptoms in varying degrees. Generally, they seem to be very restricted in host range, as is the case with the most virus diseases of animals. The mosaics are an exception, however, as they occur in a wide

range of hosts among the monocotyledonous and dicotyledonous plants, producing strikingly similar chlorotic-mottling symptoms in all. In this respect the mosaics seem to hold a position among plant virus diseases somewhat similar to that of rabies and certain poxes among the virus diseases of animals. According to Stimson (42) rabies will develop and run its course in practically all mammals. He states that fowls also are susceptible to inoculation, and that frogs and tortoises are sometimes refractory to the disease. In rabies, however, a single virus is involved; at least studies of its properties do not indicate that different viruses exist. Several of the pox diseases occurring in some mammals produce their similar but somewhat modified symptoms on different species, but in these cases it appears that different viruses are involved.

Of all the virus diseases of plants, the mosaics have received the most attention, particularly the mosaic of tobacco, partly because it was the first disease demonstrated to be caused by a filterable virus; but more probably because it is very readily transmitted by artificial means and the virus is highly resistant. Relatively little work has been done on the properties of plant viruses, and that which has been done deals largely with the viruses of the mosaics, especially tobacco mosaic. This virus is very resistant to desiccation, temperature, various chemicals, and disinfectants. Allard (1) found that it was not inactivated when subjected to temperatures of 85° and 90° C. for 10 and 5 minutes, respectively. On the other hand, the virus of cucumber mosaic (11) is inactivated in 10 minutes at temperatures above 70° C. This virus is also less resistant to desiccation and to various chemicals than that of tobacco mosaic.

It is fully recognized that certain difficulties are encountered when an attempt is made to interpret data obtained from property studies made on the mosaic viruses. These substances have not been isolated in a pure state, and therefore, for the present at least, they must be studied in association with the host fluids. Plant juices are complex in nature, and it is not known just how much influence they may exert on the behavior of a given virus when subjected to different treatments. However, even though it is granted that this situation constitutes an obstacle and that the present methods of carrying on property studies may be considered rather crude, it still appears that there is complete justification for investigating the properties of the viruses. Undoubtedly such studies will lead to the development of new and more satisfactory methods and to new interpretations.

The property of inter-host transmission of the mosaic viruses has been studied to a considerable extent by Doolittle (11, 12, 13), Walker (43), Elmer (14), Brandes and Klaphaak (6), and others, and very significant results have been obtained. Doolittle and Walker have found by direct

inoculation methods that the virus of cucumber mosaic will produce mosaic in a number of dicotyledonous hosts. Some of these hosts are closely related to the cucumber, whereas others are widely separated in relationship. Brandes and Klaphaak carried on direct cross-inoculation studies with the virus of sugar cane mosaic and found that it produces the disease on a large number of grasses but in no case on species distantly related to the cane plant. These and similar results obtained by other investigators have naturally led to the belief that there are probably several mosaic viruses, each of which may have a different host range.

In addition to making direct cross inoculations Doolittle and Walker (13) obtained infections through intermediate or bridging hosts which had been impossible to secure by the direct method. They found that the mosaic of cucumber may be transmitted to tobacco provided the virus is first passed through the pepper plant, whereas their investigations show that this cross inoculation is not successful if made directly. Elmer (14) also conducted many cross-inoculation experiments and obtained certain results which are very striking. He reports that the mosaic of sugar cane is transmitted to tobacco when the virus is treated with acetone. Without the acetone treatment he claims negative results. He also reports infection from cross inoculations between other hosts of rather distant relationship.

Some workers have failed to verify certain mosaic infections from cross inoculations which others have reported. Although some of these discrepancies appear to be caused by faulty methods, it is possible that certain of these apparent inconsistencies may be explained on the basis of an overlapping of the host ranges of viruses which are not identical in all respects.

Although the cross-inoculation studies throw considerable light on host ranges and on the differences between mosaic viruses, it appears that these studies should be coordinated with the study of other properties of viruses.

In cucumber mosaic (11) the virus is presumably inactivated when subjected to temperatures above 70° C. for 10 minutes, whereas the virus of tobacco mosaic (1) is not inactivated when held at 85° C. for the same length of time. The cucumber-mosaic virus is also less resistant to drying and retains its virulence for a shorter period than the virus of tobacco mosaic. The question now arises as to the stability of the thermal "death" point and other properties of a given virus when it is subjected to different treatments and to a direct or bridging host cross. Will the thermal "death" point of the cucumber-mosaic virus remain fixed after it passes through the pepper plant and gets into the tobacco plant, or will this property change and approach the thermal "death" point of the tobacco-mosaic virus? Will its reaction to drying or to chemicals and its retention of virulence be changed to any extent by this cross infection? The same general questions also arise concerning the sugar cane-acetone-tobacco cross infection reported

by Elmer (14). Obviously such studies necessitate systematic investigations of the properties of the viruses in conjunction with cross inoculations. Although this procedure entails considerable effort, it is evident that the data from such combined studies will be of much greater value than those obtained when no attempt is made to coordinate property studies with cross-inoculation work.

It would seem that a knowledge of the stability or instability of the properties of viruses after passage through various hosts or after subjection to other treatments will give us a better idea as to the possibility that all mosaics are due to a single virus or to several different ones. Such information may also throw some light on the nature of the viruses.

Several attempts have been made to cultivate plant viruses *in vitro*, and certain investigators claim to have obtained interesting results. Bewley (5) reports that he obtained an unusual type of growth from the virus of tomato mosaic in culture tubes, but his inoculations were not completed at the time he published and it is impossible to draw final conclusions regarding his work. McWhorter (29) applied tissue-culture methods in his studies of the cell inclusions associated with the Fiji disease of sugar cane and claims to have observed the germination of the bodies. He concludes that they are amœbæ.[4]

Recently the causal phase of the plant-virus problem has been attacked vigorously by means of histological and cytological methods. In several cases it seems that diseased cells show striking similarities to those in many animals attacked by virus diseases; in fact, this similarity appears so marked that it led Palm (34) to consider that the cell inclusions and microscopic granules associated with tobacco mosaic are Chlamydozoa-Strongyloplasmen.

CELL INCLUSIONS

As shown in table 1, cell inclusions are known to be associated with a few infectious diseases of plants, and in most instances these particular diseases are known to be caused by viruses. However, in other cases the causes are

[4] Since the preparation of the present paper Olitsky (Jour. Exp. Med., Vol. 41, No. 1, 1925) reported the successful increase of the causal agent of tobacco and tomato mosaic in fluid from tomato plants. This is the most convincing paper which has yet appeared on this phase of the plant virus problem. Regardless of the interpretation which may be placed on this increase of the virus, the demonstration that a plant virus apparently increases *in vitro* is significant in that it paves the way for the development of new methods for studying the mosaic problems. The results encourage the development of tissue culture methods and they stimulate one to make a systematic attempt to discover some green alga which may be susceptible to a mosaic virus. Such a combination would facilitate the matter of obtaining much direct microscopic evidence on many obscure processes and also enable the accumulation of much data in a simpler manner than is now possible.

of a less definite nature. So far as known, all of these inclusions occur in the cell cytoplasm. None has been found in the nucleus, as have some of the inclusions in diseased animal cells.

Iwanowski (17) appears to have found the first cell inclusion associated with a definite virus disease of plants. In 1902 he reported these bodies in the cells of the light-green areas of mosaic tobacco leaves. In these same cells he also found many small granules which he thought were bacteria. He interpreted the large inclusions as products of degeneration. In 1922 Palm (34) also found inclusions and granules in the cells of mosaic tobacco leaves, and he came to the conclusion that they were the Chlamydozoa of Prowazek (the Strongyloplasmen of Lipschütz). In 1924 Rawlins and Johnson (37) and Goldstein (15) also confirmed Iwanowski's observation.

TABLE 1.—*List of host plants and diseases with which cell inclusions have been found to be associated.*

Disease	Host	Authority[1]
Brunissure	Grape	35
Mosaic	Tobacco	15, 17, 34, 37
Fiji	Sugar cane	22, 25, 29
Mosaic	Corn	18
"	*Hippeastrum equestri*	19, 20
"	Sugar cane	21
"	Potato	39
Rosette and mosaic mottling	Wheat	28
Mosaic	*Hippeastrum johnsonii*	27

[1] See "Literature cited," p. 200.

As in the case of animal-cell inclusions, many theories are being advanced concerning the nature of inclusions in plant cells. Lyon (25) was inclined to think the bodies in the gall cells associated with Fiji disease are organisms, and McWhorter (29) claims definitely that they are amœbæ. Kunkel (22) inclines to the belief that they are organisms on account of cytological evidence which indicates that they grow and divide in the cell and that the inclusions associated with the mosaics of corn (18) and *Hippeastrum equestri* (19) are organisms. Rawlins and Johnson (37) do not attempt to interpret the cell inclusions occurring in mosaic tobacco. Goldstein (15) thinks they are not secretion or degeneration products arising from or in the neighborhood of the nucleus. She reports observations dealing with the movement of these cell inclusions which are apparently considered suggestive that the bodies may be independent organisms. The

writer has observed this phenomenon many times in the cell inclusions associated with wheat rosette. However, it has always been very difficult to differentiate with certainty between what might be a pushing out or drawing in of the periphery of the body, suggesting pseudopodia, and the rolling and tumbling movements of irregularly shaped rigid bodies when circulating about in the streaming cytoplasm. Inclusions such as those illustrated by McKinney, Eckerson, and Webb (28, pl. 6, fig. 5; pl. 7, figs. 6 and 10; pl. 8, figs. 2, 4 and 10) may assume very curious and irregular shapes when moving in the streaming cytoplasm. Smith (39), who observed amœba-like cell inclusions associated with potato mosaic, thinks these bodies are degeneration products, probably from the nucleus. Politis (35), who has done the most recent work on the brown corpuscles in the cells of the grapevine affected by the brunissure disease, thinks that these cell inclusions are not organisms, as others have believed, but are produced by a transformation of the granular mitochondria in the diseased cells.

At the time the writer published his first paper on the rosette disease (26), the cell inclusions occurring in wheat were briefly compared with those present in certain other plants. At that time the comparisons were based on published descriptions and illustrations. Since then it has been possible to study some of the inclusions occurring in plants other than wheat. Although extensive comparisons have not been made under different sets of conditions, preliminary observations indicate that some of these inclusions differ in their general structure and morphology, and there is a suggestion that all of them may not originate in the same manner. Also, their general relations to the host cells seem to differ to a considerable degree. In general, the inclusions associated with tobacco and potato mosaics, the Fiji disease of sugar cane, and the rosette disease of wheat resemble each other in form and structure, differing somewhat in their relations to the host cells. In a few cases some of these inclusions appear to be made up of what appears to be a rather finely granular matrix. However, most of the bodies possess a matrix which is strikingly homogeneous in nature. Except in the very early stages of development, the matrix of all these inclusions contains vacuoles which vary in number and size. In some cases large granules may be present in the matrix or in the vacuoles, and occasionally the large vacuoles appear to be filled with a fine granular substance. It is not uncommon to find inclusions in this group which appear to be spheres. The outer matrix, or shell, of such a sphere will contain many very small vacuoles, and there may be one large central vacuole or several smaller ones located in the interior. All of these inclusions appear to be surrounded by a membrane and to consist of protoplasm. They are strikingly similar to certain forms of Negri and Guarnieri bodies associated with rabies and smallpox, respectively.

The cell inclusions associated with corn, Hippeastrum, and sugar cane mosaic present a somewhat different appearance from the above-mentioned inclusions. Many of them seem to be made up of compactly arranged rather large, independent granules, sometimes around "vacuoles" or unoccupied areas. In many cases the writer has caused the separation of these granules by pressure and has seen no evidence that these bodies are surrounded by a membrane. In some ways these inclusions resemble the Prowazek bodies which are associated with the trachoma disease of the human eye. They are not so suggestive of protoplasm as the bodies mentioned in the previous paragraph. In the studies on the inclusions associated with *Hippeastrum johnsonii* (27) the writer finds that many of the host nuclei are shrunken and small in cells which contain inclusion bodies. Kunkel (18) observed this in corn. This condition has not been noted among nuclei in wheat cells containing inclusions.

The writer is not yet in a position to interpret the nature of the cell inclusions he has studied most intensively in the wheat plant. As in the case of the Fiji bodies (22) they increase in size, and in the few instances where two bodies have been noted in one cell there has been evidence of division; however, these phenomena do not seem to lend any more support to the theory that the bodies are independent organisms than that they may be protoplasmic aggregates arising within the cell. If these and many other cell inclusions and cell granules are independent organisms it seems rather doubtful whether the case can be proved conclusively by the present cytological methods and optical facilities. If they are organisms, the fact can best be determined through culture and inoculation studies in combination with cytological methods.

Although the cell inclusions associated with some of the virus diseases of plants resemble those in certain animal virus diseases, it is questionable whether the interpretations which have been developed on the animal side regarding the nature of inclusions and microscopic granules should be accepted too freely by plant investigators; and although several of the methods which have been used to study animal inclusions may be applicable to the study of these bodies in plants, it appears from preliminary investigations that additional methods must be developed by plant workers. Giemsa's method of staining has been used widely in the study of animal-cell inclusions, and the conclusions of several workers are based to a large extent on results obtained with this stain. However, the writer's experience with this stain in plant tissues has not been very satisfactory. After more experience with it dependable results may be obtained, but at present the writer is inclined to agree with Dobell (10) that the value of this method has probably been somewhat overestimated.

From the observations made thus far it seems thoroughly worth while to determine the extent of the association of cell inclusions with plant-virus diseases, especially in the case of the mosaics. The writer's experience indicates that cell inclusions may not be associated with all of the mosaics; or, if so, they may not be abundant in all cases. To date, it has not been possible for him to find definite inclusion bodies associated with the mosaics of cucumber, raspberry, or sweetclover. However, these negative findings can not be considered as final, as he has frequently had difficulty in finding very many cell inclusions in some of the mosaic corn, sugar cane, and Hippeastrum grown in this country. Had it not been known that inclusions occur in these hosts, doubtless they would have been overlooked in some of the material examined. It may be that the range of favorable environmental conditions for the occurrence of cell inclusions in some hosts is more limited than that for typical mosaic mottling. It would be of great interest to know something of the inclusion phenomenon which exists in the sugar cane-tobacco mosaic cross which Elmer (14) claims to have obtained. Cell inclusions are associated with both of these diseases, and as these particular inclusions seem to show different characteristics it is important to know whether the cane virus produces the cane type or the tobacco type of inclusion in the tobacco plant. Likewise it is important to determine the behavior of inclusions in the cucumber-pepper-tobacco cross. Such observations should bring out correlations between cell inclusions and the hosts or the viruses which will throw additional light on the probable nature of inclusions and on the specificity of viruses.

One of the striking results which comes out of a general study of the virus problem is that no single lead or line of attack stands out as the one most likely to lead to the determination of the exact nature of the viruses. On every hand the evidence shows that no matter which single line of attack is followed, the limitations of present methods seem to prevent the attainment of the facts most desired. To the writer it appears that there is complete justification for piling up indirect as well as direct evidence on all phases of the virus problem. It appears also that many of the single lines of study should be coordinated and new experiments should be carried out in order that we may obtain a better idea as to the adequacy of some of the experimental methods in use and so that many of the theories which have been advanced may be considered from new angles.

A systematic study should be made of the environmental factors which may influence the various phases of these diseases. Soil and air temperature studies have been carried out with a few hosts and it is interesting to note that the mosaic diseases appear to be most injurious at temperatures which are seemingly most favorable to the development of the host plants.

This general relationship seems to be rather unusual and one is naturally led to inquire as to its possible significance. It would be of considerable interest to know how general this relationship may be among the mosaics and other virus diseases. With the increasing tendency on the part of workers to give consideration to the idea that some component of the protoplasm of the healthy host may become the disease-producing agent or virus, it appears that more time should be devoted to the study of healthy plants grown under various environmental conditions. In considering this theory it would seem that some irregularity in the hosts' environment must play an important part in causing the first upset which results in the development of a disease producing virus, and it appears that the only way to prove the theory beyond doubt is to determine the conditions which may cause this supposed first upset in the healthy host. The effect of ultraviolet light on the vitamin content of many foods thought to be devoid of certain of these substances is a case not wholly unlike the one under consideration.

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QUANTITATIVE STUDIES ON THE EFFICIENCY OF FUNGICIDES

JEAN MACINNES[1]

WITH NINE FIGURES IN THE TEXT

Considerable information has accumulated with reference to the efficiency of various substances both for disinfection and for the control of plant diseases. The results obtained have been largely empirical, however; and, although attempts have been made to define the factors which make one substance an effective killing agent and another a poor one, only a few investigators have made use of quantitative physical and chemical measurements for this purpose. Among these investigators may be mentioned Osterhout (4), Brooks (1), and Shearer (2, 3). In the experiments described in this paper, such measurements were made with the hope of obtaining some definite information regarding the subject of toxicity and also with the hope of developing a rapid method of comparing the effectiveness of fungicides.

THE APPARATUS

The apparatus which was adopted for making the measurements consisted primarily of three glass cells, a diagrammatic representation of one of which is given in figure 1. One electrode (F) was fused into the base of the cell, the other (C) being inserted from above. The organism was placed between two perforated hard-rubber disks (D and E), and inside a cavity made by disks of soft-rubber (H). Fresh solution was constantly perfused through the organism by means of the tube (N) entering at the base of the cell.

The three cells were fastened securely on a stand and remained there throughout each experiment. The upper electrodes were fastened on the stand in such a manner that they could always be replaced in exactly the same position in relation to the lower electrodes. They were also arranged so that the distance between them and the lower electrodes was approximately the same for all three cells. In this way it was possible to have the initial resistance of the three cells, before the organism was placed in them, very nearly the same.

[1] The author wishes to express her appreciation of the assistance and encouragement given her by Professor Prescott, of the Department of Biology and Public Health of the Massachusetts Institute of Technology, and Professor Osterhout, of the Department of Botany of Harvard University.

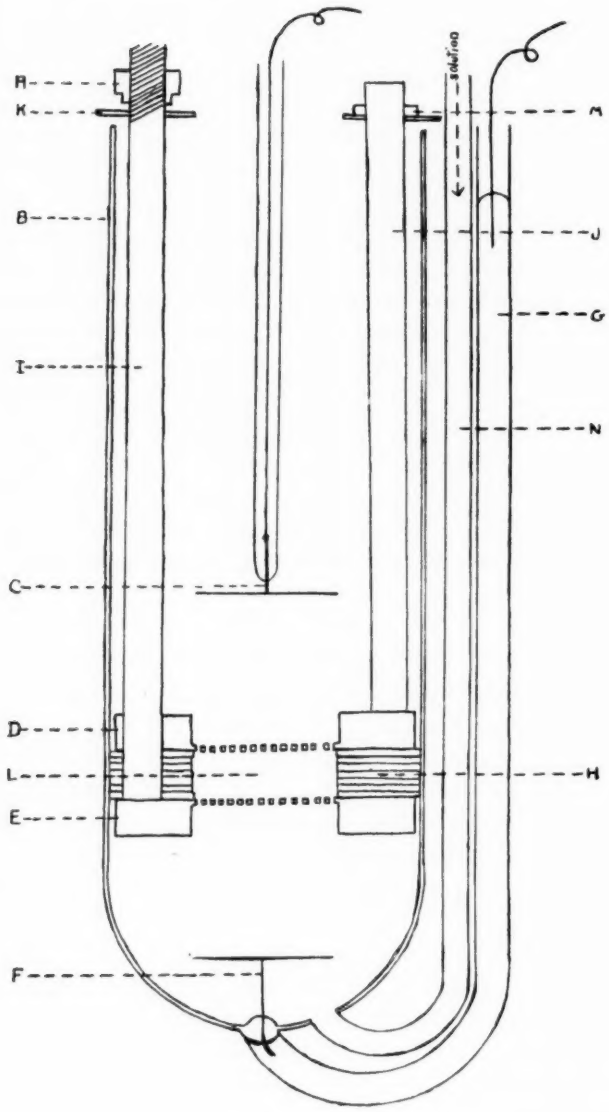

FIG. 1. Diagrammatic representation of one of the cells in which the conductivity measurements were made. A. Bolt for adjusting the position of the disks. B. Wall of the glass cell. C. Upper electrode. D. Upper hard-rubber disk. Note perforations for passage of the current and the solution. E. Lower hard-rubber disk. F. Lower electrode—fused into base of cell. G. Tube filled with mercury for making connection with

The position of the hard-rubber disks in relation to each other had to be controlled very carefully. In order to do this, three rods of hard-rubber were attached to the upper side of each, the rods of the lower disk (see fig. 1) (E) passing through the upper (D) in such a manner that they alternated with those of the upper. All six rods terminated at the top of the cell, but those coming from the upper disk were fastened to a metal ring (K), and the others passed through holes in the ring and were threaded for bolts (A).

As it was impossible to fit these hard-rubber disks so tightly that none of the current passed between the edge of the disk and the glass, similar disks of soft-rubber (H) were made. They were slightly greater in diameter than the hard-rubber ones but were less than an eighth of an inch in thickness, and, instead of being perforated, had holes three quarters of an inch in diameter cut in their center. Seven such disks were superimposed on the lower hard-rubber disk, the rods from the latter passing through holes in the soft rubber made for that purpose. When the upper disk was put in and the bolts screwed into place, the soft-rubber expanded and pressed against the cell sufficiently to prevent the passage of current through that part of the cell, a cavity of about a half an inch in depth and three quarters of an inch in diameter being formed in the center. The organism was stuffed into this cavity.

The solution which entered at the base of the cell through the tube (N) passed up through the perforations in the lower disk, through the organism, through the upper disk, and was drawn off by means of suction. The soft-rubber disks prevented it from passing between the walls of the cell and the disks. The solution, before entering the cell, was conducted through the water bath in glass tubes from a large bottle held about two feet above the apparatus. A constant rate of flow of the solution was adopted which was shown to be sufficient to keep the resistance of the solution the same throughout the time that the experiments were being carried out.

The temperature of the water bath in which the whole apparatus was immersed was $25 \pm .1°$ C. Figure 2 is a diagrammatic representation of the whole apparatus.

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lower electrode. H. Soft-rubber disks. I. Rod of hard-rubber coming from lower hard-rubber disk. J. Rod from upper hard-rubber disk. K. Metal ring through which the rods pass and upon which the bolt (A) presses when the disks are in position. L. Cavity in which the organism is stuffed. M. Bolt for holding metal ring to the rods which come from the upper disk. N. Tube through which the solution passes before it perfuses through the organism.

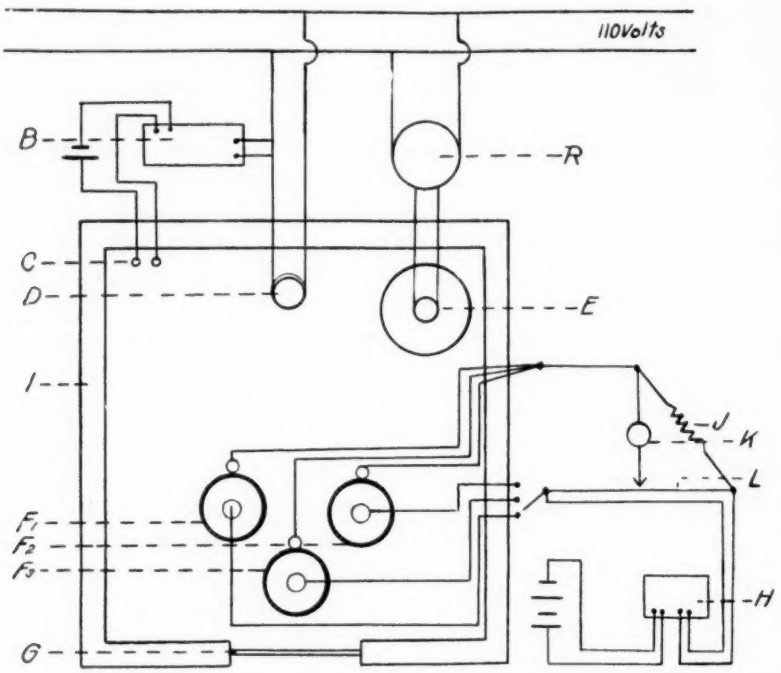

FIG. 2. A diagrammatic representation of the whole apparatus. R. Motor for running the stirrer. B. Relay for controlling the lamp which heated the water bath. C. Mercury thermo-regulator. D. The lamp which heated the water bath. E. The stirrer. F_1, F_2, F_3. The glass cells with their connections to the bridge. G. Window in the tank. H. Buzzer. I. The tank which held the water for the water bath. J. Known resistance on the bridge. L. The slide wire on the bridge. K. The telephone receivers.

THE ORGANISM

The organism used in all of the experiments here to be described was *Aspergillus niger*.

CULTURE MEDIA AND METHOD OF MAKING CULTURES

The medium in which the organism was grown was essentially that which is known as Czapek's solution. It was used four times as strong as recommended by Czapek, however, as it was necessary in these experiments to have a solution of moderately low resistance. The spores of the organism were produced on slants of potato dextrose agar and then inoculated into flasks of 750 cc. capacity containing 100 cc. of the modified Czapek's solution. After being at a constant temperature of 24° C. for about three days, an even growth of the organism was obtained on the surface of the medium.

THE TOXIC SOLUTIONS

The killing agents used in these experiments were mercuric chloride, copper sulphate, and formaldehyde. These substances were added directly to the modified Czapek's solution, the same as was used for growing the organism except that the KH_2PO_4 was omitted.

THE PROCEDURE

Material, grown as described above, was carefully removed from the flasks in which it had grown in such a manner as to prevent tearing or breaking. Each sheet of the mycelium was then cut into three equal parts, and one of each, including some of the culture solution in which the fungus had grown, was immersed in the liquid in one of three suction flasks and the whole put under suction for about five minutes in order to remove a large part of the air held between the cells. In this way, each cell was supplied with an equal amount of exactly the same material.

In order to determine the resistance of the cell without the presence of the organism, the cell was filled with the solution to be used in the experiment, the upper disks put in, the bolts screwed down, and the upper electrode set in place. When the soft-rubber disks pressed against the walls sufficiently to prevent any of the current from passing through that part of the cell, a maximum resistance was obtained. A little experience made it possible to determine this point merely by noting the tightness of the screws.

When the resistance of the solution had been measured, the upper disk was removed and the organism was stuffed gently into the cavity made by the soft-rubber disks. After replacing the upper disk and setting the electrode in place, the resistance of the whole was taken. Perfusion was started at once.

The organism was put directly into the killing agent and the initial resistance obtained considered to be the resistance of the normal organism before it had been affected by the toxic substance. In calculating the results and plotting the curves, this figure was called 100 per cent and the percentage rise or drop in resistance obtained, due to the action of the killing agent, was based on this figure.

CONDUCTIVITY EXPERIMENTS

Before it was possible to carry out the experiments by which the change in conductivity due to the various killing agents could be compared, it was necessary to make a number of preliminary experiments. It was necessary to know, for instance, the changes which might take place in the organism without the presence of the killing agent. Experiments were

carried out in exactly the same manner as in all the other experiments, except that the modified Czapek's solution alone was used. Under these conditions, the resistance of the organism remained constant, often as long as three hours. Owing to the accumulation of carbon dioxide produced by the organism, however, the resistance of the organism eventually rose rapidly and never dropped again to the original amount. This fact made it impossible to compare the normal organism directly with that treated with the toxic substance. As these results were totally different from those obtained when the toxic substance was present, however, it was not necessary in this case to base the comparisons on the normal.

It was found that the initial resistance of the organism from one day to the next varied considerably, owing, partly at least, to the fact that it was impossible to inoculate the solutions with exactly the same number of

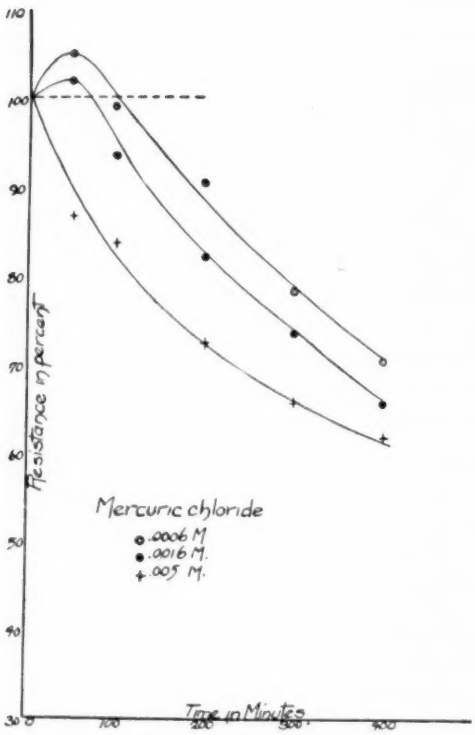

FIG. 3

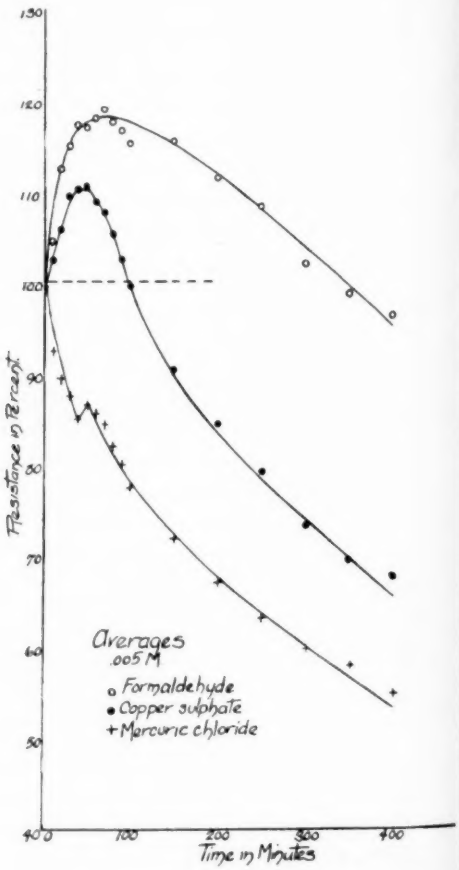

FIG. 4

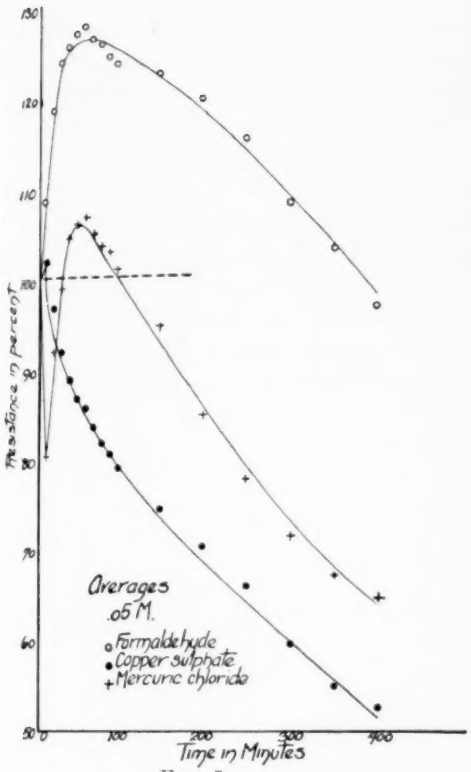

FIG. 5

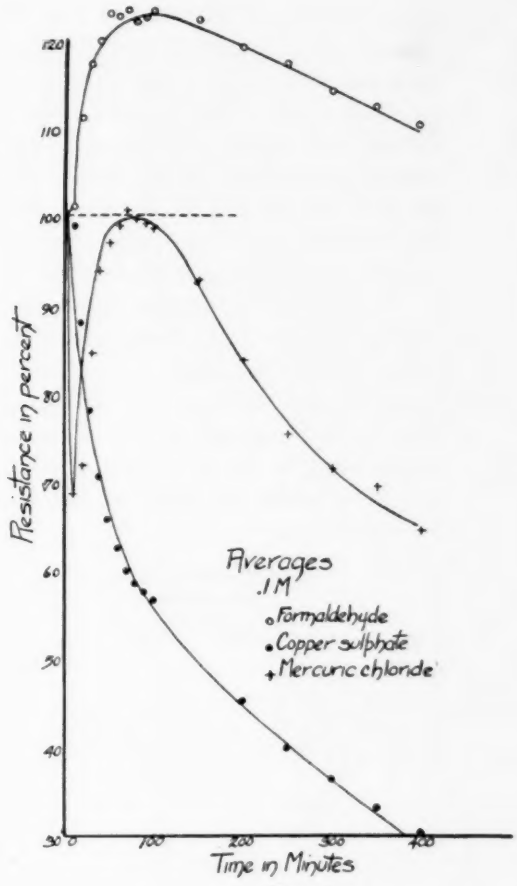

FIG. 6

spores each day. This caused a variation in the quantity of material which developed from day to day, and consequently in the quantity of material which was put into the cells. As the results were all used on a basis of percentage of the original resistance, however, these variations were not of great importance.

In order to determine the extent to which the resistance of the living organism might be changed upon death, typical samples were killed by heat and by the use of copper sulphate. In both cases, the resistance after death was found to be about 25 per cent of what it was when the organism was living. Later experiments indicated that, when it was killed with formaldehyde, the drop was not so great.

When material which had been killed was subsequently treated with the killing solutions in the same manner as the living organism was treated, no

such changes as occurred in the latter case were ever obtained. Such changes in resistance as occur with the living organism, therefore, must be a function of the living cell.

With these factors in mind, and after proving that the three cells could be counted on to give comparable results, a series of experiments were conducted in which different concentrations of mercuric chloride were compared. The results are given in figure 3. It is evident that an increase in concentration of the killing agent increases the speed with which the resistance of the organism changes.

Several series of experiments were then carried out in which equal concentrations of the three killing agents were compared, each cell being supplied with an equal amount of identical material, the only variation being that of the toxic substance. In one series, the concentration was .005 M., and later .05 and .10 M. were made use of. The results are given in figures 4, 5, and 6. These same results are made use of in figures 7, 8, and 9, in which the different concentrations of the three toxic substances are compared.

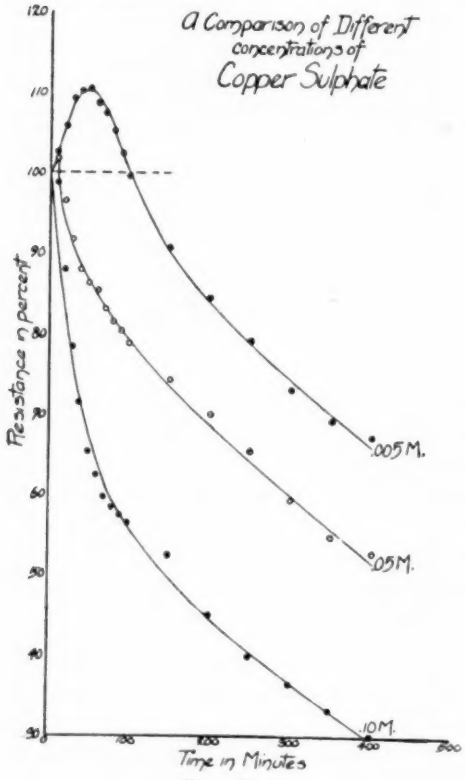

FIG. 7

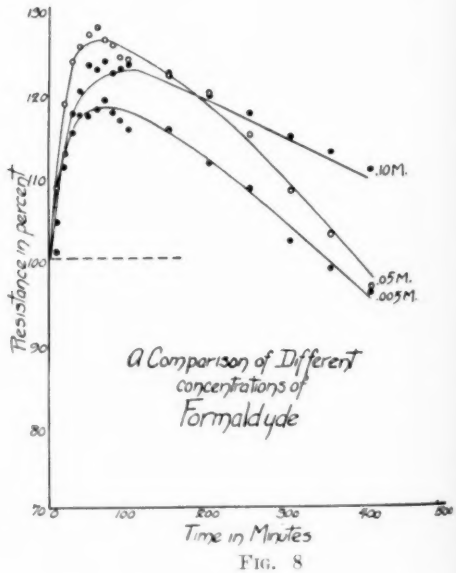

FIG. 8

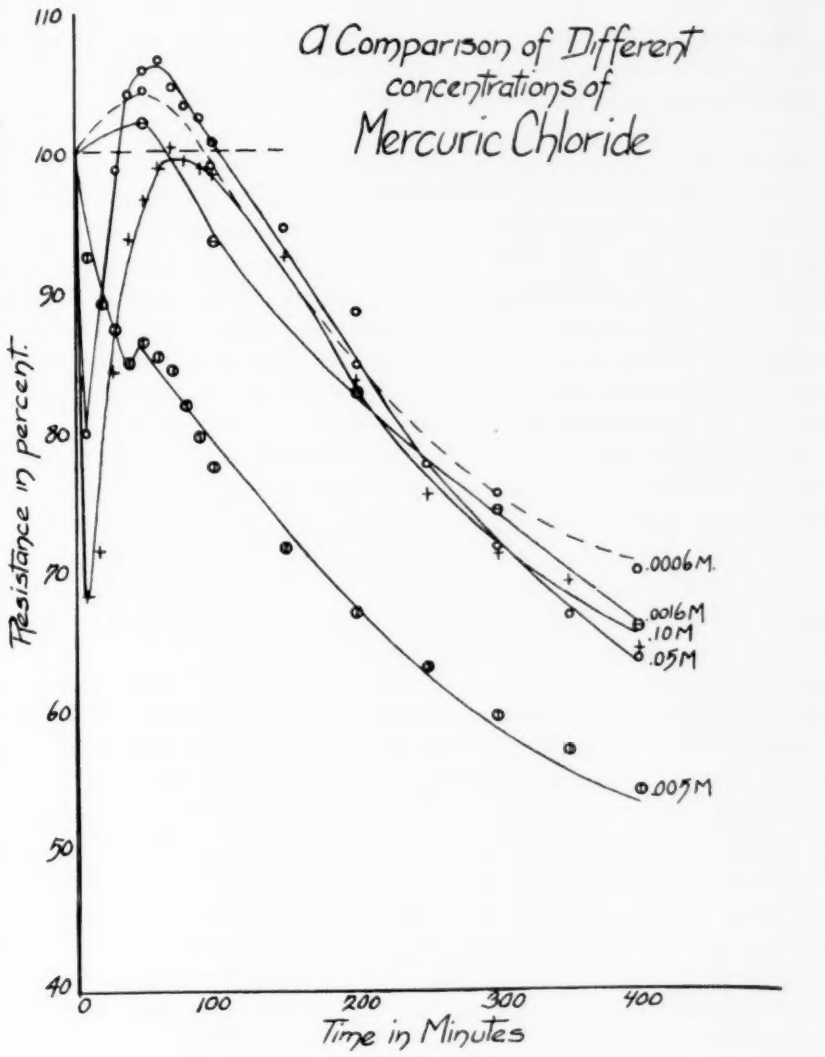

FIG. 9

An attempt was made to find out whether a direct relation could be found between the length of exposure necessary to prevent growth of the organism and the curves obtained for the three killing agents. It was found that mercuric chloride was much more effective in preventing growth of the fungus than either formaldehyde or copper sulphate; and, of the latter, formaldehyde was more effective than copper sulphate. No growth was obtained when the organism had been exposed to mercuric chloride

longer than one minute, when the concentration was only .0006 M. The material withstood copper sulphate of .10 M. for more than 200 minutes, but material treated with formaldehyde did not grow if it had been exposed more than 10 minutes to this same concentration.

DISCUSSION

From an examination of the results obtained it is evident that, if electrical conductivity is a measure of the death process, the whole problem is very complicated and that the changes taking place as death is brought about vary with the different ways of killing. It is quite probable that more than one process is being measured. However, a number of definite relationships hold true, and it is fairly safe to conclude that some notion of the relative efficiency of the toxic agents for fungi may be obtained by measuring the changes in electrical resistance.

The results with copper sulphate (Fig. 7) show quite clearly that a direct relationship exists between concentration and changes in resistance. The rate of decrease in resistance with .10 M. copper sulphate is much greater than with .05 M. and very much greater than with .005 M. As a strong solution of copper sulphate would be expected to kill more rapidly than a weak one, it would seem that resistance may be used as a measure of the effectiveness of copper sulphate as a fungicide.

When mercuric chloride was the killing agent, the results were much more complicated. The three low concentrations (Fig. 3) indicate again that the high concentration kills the organism more rapidly than the lower ones. As the concentration is increased (Fig. 9), however, other processes come into play. The very striking decrease in resistance, followed by a similarly striking increase where the strong solutions were used, is difficult to explain. However, if only the first part of the curves for all the concentrations of mercuric chloride are taken into consideration—the first ten minutes, for instance—the relative effectiveness of the various concentrations in decreasing the resistance is evident. The experiments on growth indicated that the cell is probably damaged beyond repair before the subsequent rise in the strong concentrations begins. On this basis, resistance may be used as a measure of the effectiveness of mercuric chloride in killing the organism as well as for copper sulphate.

The results with formaldehyde (Fig. 8) differ so radically from those obtained with other substances that they must be interpreted on a different basis. The curves all show an initial rise, followed by a gradual drop, which eventually falls to a point somewhere below the normal line, but generally not so far below as in the other cases. Osterhout (4, page 107) has shown that, at least in the case of *Laminaria*, the organism is injured when the

resistance is above the normal as well as when it is below. In all probability the same is true with this fungus when it is treated with formaldehyde. It seems probable that an increase and a decrease in resistance both may be used as a basis for determining injury. Some experiments with strong formaldehyde tended to confirm this theory. Further experiments in which different concentrations of formaldehyde are compared, and experiments on recovery similar to those which have been done on *Laminaria*, should be carried out to test this point.

The results obtained for these three toxic agents with *Aspergillus* indicate rather definitely, therefore, that, with this particular fungus at least, conductivity may be used as a measure of the relative effectiveness of fungicides. It would be extremely interesting to find out whether other fungi would behave similarly and whether other toxic substances would give similar types of curves. If these things were true, this would be a very valuable method of determining, rapidly and perfectly definitely, the relative toxicity of fungicides.

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BIOLOGICAL AND CULTURAL STUDIES OF EXOASCUS MIRABILIS

A. J. Mix

With Two Figures in the Text

In a recent paper (2) an account was given of some studies on the biology of *Exoascus deformans* (Berk.) Fuckel and on the behavior of that fungus in pure culture. The present report is concerned with similar studies of *Exoascus mirabilis* Atkinson. As the methods employed were the same as those used with *Exoascus deformans*, a detailed discussion of them will be omitted, and a brief account will be given of the principal results obtained.

THE FUNGUS AND ITS EFFECT ON THE HOST

Exoascus mirabilis is common in eastern Kansas, causing deformation of the shoots of the Chicasa plum, *Prunus angustifolia* Marshall. The characters of the fungus and the symptoms of the disease produced are well described by Atkinson (1) and the description will not be repeated. A deformed shoot is shown in figure 1.

The disease makes its appearance in Kansas late in April, usually one or two weeks later than peach leaf curl. Mature ascospores may be found the last of May or the first of June, these appearing a little later than the ascospores of *Exoascus deformans*.

Twigs and leaves of the current season's growth are affected, the hypertrophy never extending back into the wood of the previous year. Terminal shoots are more frequently affected than laterals. This may be explained, at least in part, by the habit of growth of the tree, since lateral buds usually produce short spurs, and seldom develop into rapidly elongating shoots. Young trees, one to three feet high, are more severely infested than older trees. The reason for this is not apparent.

Soon after the formation of ascospores the affected part of the twig is overrun with saprophytic fungi, and a little later it shrivels and dies. These dead and dried-out twigs may be observed on the tree for a year or more. The amount of injury to the tree is not great, and, since the host has no economic importance, the disease cannot be said to have any.

No affected fruits of *Prunus angustifolia* have been found, although Atkinson (1) describes deformation of fruits of this species by *Exoascus mirabilis* var. *tortilis*.

ISOLATION OF THE FUNGUS

Isolations were made from ascospore-bearing material in 1922 and again in 1924. Isolations from the interior of diseased twigs were made in 1922 and 1923. The number of successful isolations of the latter type was thirty out of a possible ninety-six, indicating that the fungus is somewhat more readily isolated from the interior of diseased tissues than is *Exoascus deformans*. The total number of cultures under observation during these studies was fifty-nine.

FIG. 1. A Chicasa plum shoot deformed by *Exoascus mirabilis*. Infection occurred early in the season.

BEHAVIOR OF THE FUNGUS IN CULTURE

The fungus closely resembles *Exoascus deformans* in habit and character of growth in culture media, the only distinction between the two being a very slight difference in color of the colony. When both organisms are grown on the same media the colonies of *Exoascus mirabilis* show a little more pink than those of *Exoascus deformans*. This color difference can-

not be definitely expressed: colonies of both organisms on potato dextrose agar approximate 9″ f, pale vinaceous pink, of Ridgway's color chart, but their color really lies between that and white.

Favorable media for the growth of *Exoascus mirabilis* are: potato dextrose agar, potato dextrose gelatin, potato dextrose broth, sweet potato broth; sweet potato, carrot, and beet plugs; bean pods. Sweet potato plugs seem to favor the formation of the so-called resting cells of the fungus, and bean pods are favorable to the formation of actively budding cells, germ tubes, and mycelia. Media supporting less growth than those named are: potato maltose agar, string bean agar, and steamed rice. Potato sucrose agar gives very restricted growth. Plugs made from old potatoes, which may be believed to contain appreciable amounts of sugar, have been found to support nearly as much growth as other vegetable plugs. On plugs prepared from new potatoes almost no growth occurs. The fungus refuses to grow on beef agar, beef gelatin, and in beef broth. No synthetic media have been tried.

MORPHOLOGY IN CULTURE

Cells formed in culture are for the most part oval "conidia" which multiply by budding. In size these conidia range from 2.4 x 4.0 to 5.2 x 8.0 microns. In some cultures, especially in those on bean pods, there occur conidia which are oblong in shape. When the culture is not actively growing, budding is interrupted and the conidia then appear as oval cells with vacuolate protoplasm. So-called resting cells are also formed. These are round, oval, or oblong, and possess thicker walls than the conidia. In size they range between 4.0 x 4.8 and 12.0 x 17.8 microns. They germinate by rupture of the wall and the emergence of a thin-walled cell, and, as was suggested in connection with *Exoascus deformans*, it seems possible that they may represent ascogenous cells.

Germ tubes and short mycelia are also found in culture. These latter are occasionally several cells long and often quite irregular in diameter.

Finally, it should be stated that there are no observable microscopic characters by means of which cells from cultures of *Exoascus mirabilis* can be distinguished with certainty from those of *Exoascus deformans*.

TEMPERATURE RELATIONS IN CULTURE

Some study was made of the effect of temperature on the rate of growth of this organism in culture. Incubators adjusted to cover the entire range of growth were not available. It was possible, however, to learn something of the more important temperature relations. In table 1 are given data on growth of *Exoascus mirabilis* on plates of potato dextrose agar subjected to

different temperatures. Rate of growth was determined by measuring the diameter of the colonies. No attempt was made to measure colonies more closely than to one-half millimeter. The values given in the table are averages of measurements of twenty colonies representing ten separate cultures of the organism.

TABLE 1.—*Growth of Exoascus mirabilis on potato dextrose agar at different temperatures*

Temperature	4–8° C.	8–12° C.	17–21° C.	25° C.	30° C.	35° C.
First test.						
Diameter of colony in millimeters.						
Ten days	3.4	3.8	5.0	4.6	1.4	...
Second test.						
Diameter of colony in millimeters.						
Five days	.....	3.3	6.0	4.5	2.2	0
Third test.						
Diameter of colony in millimeters.						
Five days	.....	.....	.....	.....	1.3	0

From an examination of this table it is evident that the minimum temperature for growth is below 8° C., the optimum in the neighborhood of 20° C., the maximum slightly above 30° C. These temperature relations agree with those previously found for *Exoascus deformans*, except that no growth of that organism occurred at 30° C. In the third test reported in table 1, cultures of *Exoascus deformans* were placed at temperatures of 30° C. and 35° C. along with those of *Exoascus mirabilis*. The cultures of *Exoascus deformans* failed to grow. This confirms the results already reported for that fungus (2).

DEVITALIZATION OF CULTURES BY HIGH TEMPERATURES

Six vigorous agar cultures of the organism were placed in incubators at 30° and 35° C. Daily transfers from these cultures to fresh tubes of potato dextrose agar showed that one culture was killed by six days exposure to 30° C., two more in seven days, one more in nine days, and the remaining two in ten days. This indicates that, although some growth may occur at 30° C., prolonged exposure to that temperature is fatal, and the critical temperature for the organism must be near 30° C. All cultures were killed by twenty-four hours' exposure to 35° C. It was previously found (2) that exposure for eight days or less to a temperature of 30° C. was sufficient to kill cultures of *Exoascus deformans*.

RESISTANCE TO DESICCATION

A test was made to determine the length of time that cells from culture would resist desiccation on glass. The details of the method have been

previously described (2). It was found that the organism remained viable as long as 300 days when the glass slips bearing the film of dried cells were kept in the refrigerator at approximately 10° C.; that it survived similar exposure at room temperatures (20–25° C.) for 210 days, and at 30° C. for 160 days. This is evidence of considerable resistance to desiccation on the part of conidia of *Exoascus mirabilis*, and it is also apparent that desiccated conidia withstand a high temperature (30° C.) much better than conidia actively growing in culture media.

ACID-ALKALI RELATIONS IN CULTURE

The fungus was grown in plates of potato dextrose agar to which different amounts of five per cent or fifty per cent lactic acid, or of N/10 or N/1 NaOH had been added. Growth occurred on agar at pH 2.6, at pH 11.3, and at all intermediate concentrations tried. No growth occurred at pH 2.5 or at pH 11.6. Maximum growth occurred at pH 4.0 to pH 4.2.

The fungus was also grown in flasks of potato dextrose broth in which the hydrogen-ion concentration was varied by the addition of lactic acid or of sodium hydroxide in the same fashion as with the agar. Growth occurred in solutions whose initial hydrogen-ion concentrations were pH 2.81 and pH 10.45 respectively, and at all intermediate concentrations tried. No growth occurred at pH 2.69 or at pH 10.62.

Changes in the reaction of the broth during the first twenty days of growth were in the direction of increased acidity, except that solutions with initial hydrogen-ion concentrations from pH 3.0 to pH 4.0 remained unchanged during this period.

The data from which the conclusions just stated were derived will be presented in a later paper which will report a more complete study of the hydrogen-ion relations of *Exoascus mirabilis* and of *Exoascus deformans*.

INOCULATIONS

No extensive inoculation experiments have so far been performed. In 1923 the dormant buds on several twigs of *Prunus angustifolia* were sprayed with formaldehyde solution, 1 to 200. A few days later, when the tips of the buds showed green, inoculum from agar cultures of *Exoascus mirabilis* was inserted into the tips of the buds. No diseased shoots developed from any of these buds. In 1924, soon after the buds had opened, the young tips of several shoots were smeared with inoculum from agar cultures. The weather was unfavorable for infection and remained so for several weeks. The few diseased shoots that were finally found were thought to be due to natural infection.

OVERWINTERING AND INFECTION

The possession or lack of an overwintering mycelium remains an open question for several members of the Exoascaceae. No search for overwintering mycelium of *Exoascus mirabilis* has been made for the reason that diseased shoots invariably die, and it has not seemed worth while to look for such mycelium in the older, apparently healthy portions of the branches. The diseased shoots of the current season occur with no relation to those of the previous year, which is not what might be expected if mycelium remained alive in the neighborhood of diseased shoots throughout the year.

The spraying experiment reported below has a bearing on this question. On March 9, 1923, six trees of *Prunus angustifolia* were sprayed thoroughly with lime-sulfur solution, 1 to 20. Only one diseased shoot developed on any of these trees during the season. Counts made on June 7 on three nearby trees of approximately the same size as the sprayed trees gave a total of forty-two diseased shoots. This indicates that infection must be due to some overwintering spore-form, and that if overwintering mycelium exists it is a negligible factor in perpetuating the disease. What this overwintering spore-form may be is of course unknown, but it is evident that the conidia derived from budding ascospores possess two faculties: resistance to desiccation and the ability to live saprophytically, which should enable them to survive long enough to cause infection.

The following observations have a bearing on the questions of overwintering and infection. In this part of Kansas the early spring of 1924 was unusually dry. Rain occurred during the night of March 27, and there were showers the following day. This rain was insufficient to constitute what we may assume from our meager knowledge of infection phenomena in the Exoascaceae to be a good infection period, that is, a period of several hours during which the twigs and buds remain wet. With the exception of light showers on April 25, there was no more rain until April 28. During that night and the succeeding day, there occurred a prolonged rain, sufficient to constitute a good infection period, as described above.

Two twigs of the wild plum deformed by *Exoascus mirabilis* were found on April 25, and during the next two weeks careful search of a large thicket brought the number of diseased twigs observed up to eight. In a normal season the disease would have been abundant at this time. Discounting the possibility of perennial mycelium in these twigs, the disease must have resulted from infections occurring on March 28, and its scarcity may be attributed to the inadequacy of that infection period.

Following the rain af April 29, the weather remained fair until the latter part of May when several rains occurred. During the first week in June, many diseased shoots of the wild plum were found. This outbreak

of the disease was quite as severe as that which may be observed earlier in a normal season. It is worth noting that the time between the supposed infection period and the discovery of the disease is the same for each outbreak, approximately four weeks. It is probable that diseased specimens might have been found a few days earlier in each case.

That there were two definite outbreaks of the disease in 1924, corresponding to two possible infection periods, is confirmed by the following observations. On June 10 the greater number of the diseased shoots of the second outbreak were bearing ascospores. In contrast to this, the few diseased shoots which had been located earlier were past this stage and were being overrun and killed by saprophytic fungi.

There was a further significant difference between the diseased shoots observed early in the season and those found later. The former exhibited the usual form of the disease, normal elongation of the shoot being pre-

FIG. 2. A Chicasa plum shoot deformed by *Exoascus mirabilis*. Infection occurred after the shoot had made considerable growth.

vented and the deformation involving the whole shoot or all but a small basal portion. This appearance is shown in figure 1. It may be explained on the assumption that infection occurs soon after the unfolding of the bud. In marked contrast were the diseased shoots found in early June. Below the deformed terminal portions were several healthy internodes, as though infection of the growing tips had occurred after the shoots had made considerable growth in length. This appearance is shown in figure 2.

The observations reported above seem to warrant the following assumptions regarding the life-history of *Exoascus mirabilis*:

1. Overwintering mycelium, if such exists, must be a negligible factor in the incidence of the disease in the spring.

2. Infection must be due to some overwintering spore-form.

3. Infections may occur over a considerable period in the spring, depending on the frequency of infection periods.

4. Only the tender growing portion of a shoot is susceptible, the fungus being unable to deform the older, more woody part.

SUMMARY

A disease of the Chicasa plum, *Prunus angustifolia* Marshall, caused by *Exoascus mirabilis* Atkinson, is common in eastern Kansas.

The fungus has been isolated from the ascospore-bearing surfaces and from the interior of diseased shoots.

Colonies formed in culture are not easily distinguishable from those of *Exoascus deformans*.

Cells formed in culture are: budding conidia, resting cells, and short mycelia.

The minimum temperature for growth in culture is below 8° C., the maximum slightly above 30° C., the optimum near 20° C.

Cultures are killed by an exposure of ten days' duration or less to a temperature of 30° C., and by an exposure of twenty-four hours to 35° C.

Cells from culture resist desiccation on glass at 10° C. for 300 days, at 20–25° C. for 210 days, at 30° C. for 160 days.

Growth limits on potato dextrose agar are pH 2.6 and pH 11.3. Maximum growth occurs at pH 4.0 to pH 4.2.

In potato dextrose broth, growth occurs between pH 2.81 and pH 10.45. Growth of the organism induces increased acidity in potato dextrose broth cultures during the first twenty days, except that cultures between pH 3.0 and pH 4.0 remain unchanged.

Attempts at artificial inoculation have so far failed.

The disease may be controlled by dormant spraying. From this fact, and from observations on infection and progress of the disease, it is deduced

that overwintering mycelium is lacking or unimportant, that infection is due to overwintering spores (perhaps conidia), that infections may occur over a considerable period in the spring, and that only the growing tips of shoots are susceptible.

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BLOSSOM-END ROT OF PEPPER (CAPSICUM ANNUUM L.)

B. B. HIGGINS[1]

WITH FIVE FIGURES IN THE TEXT

Like the tomato and some other fleshy fruits, the pepper is subject to a physiological rot or spotting of the blossom end of the fruit, which, so far as I can learn, has previously been mentioned only once (3).

The disease is first manifested by one or more slight depressions at or near the blossom end of the half-grown fruit (Fig. 1). The depressed spot

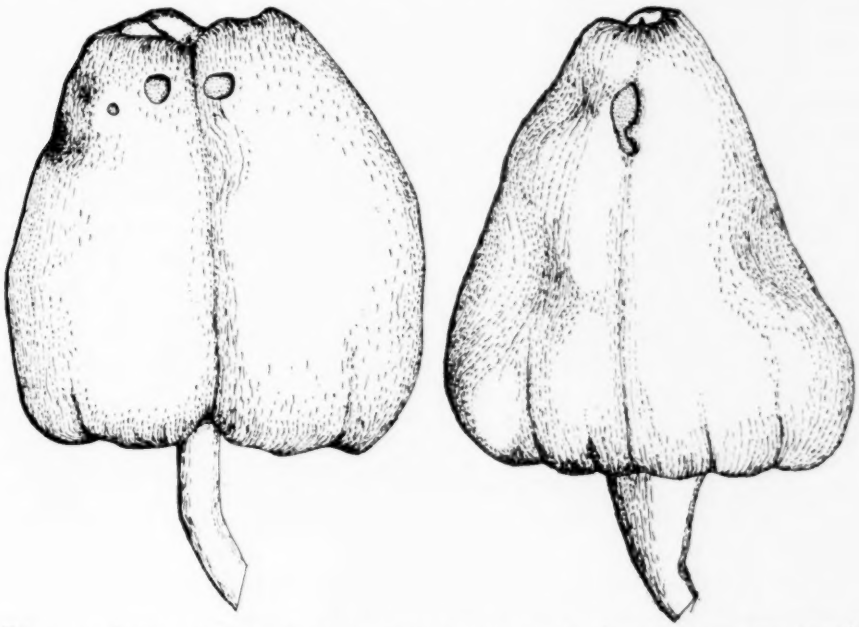

FIG. 1. A, Sketch of Pimiento pepper fruit showing single blossom-end rot spot fourth day after disease became visible (natural size). B, Sketch of Bell pepper fruit showing location and size of blossom-end rot spots second day after disease became visible.

is usually slightly paler than the surrounding healthy tissue, but sometimes becomes darker green with a water-soaked appearance. The spots enlarge to some extent, but, unlike the tomato blossom-end rot spots, never involve the entire blossom end of the fruit, unless they become infected and enlarged by microorganisms.

[1] Paper number 18, Journal series, Georgia Agr. Exp. Sta.

In the field, infection by fungi and bacteria usually occurs, and after a few days the spot may have all the appearance of having been produced by the invading organism. In Georgia the most common and conspicuous invader of the spots is a species of Alternaria; and, from reference in plant disease literature, one may infer that this is true in other regions. In some cases as high as 45 per cent loss has been attributed to an Alternaria or Macrosporium rot; but, apparently no one has ever succeeded in producing a similar rot by inoculating with the fungus reported as the causal organism.

While studying this so-called Alternaria rot in 1922, the true nature of the origin of the spots was first suspected. During that year heavy rains occurred throughout May and early June. Pepper plants, after being set in the fields, grew rapidly and, by the end of the rainy period, many had set one to five fruits. During the latter part of June and throughout July very little rain fell. No fruit rots had been found during the rainy period. I was therefore much surprised to find during the succeeding very dry weather a large percentage of the fruits decaying at the blossom end. In one field approximately 90 per cent of the early fruits became spotted.

A large number of these spots were removed aseptically and the interior diseased tissue plated out on various media. Some platings yielded cultures of various organisms, but cultures from very young spots remained sterile.

With the exception of one species of *Alternaria* (probably *Alternaria solani*), none of the organisms isolated were found capable of infecting sound pepper fruits. Under very favorable conditions this organism was able to infect sound young fruits, but only very small spots, one to two millimeters in diameter, were produced. These spots in no way resembled the spots occurring in the field.

From these observations and from comparison with analogous diseases of tomato and other fleshy fruits, it was concluded that the disease was of purely physiological origin and was closely associated with the water relations of the plant.

The conclusion was further strengthened by field observations in 1924. In a one-acre field on the Station farm, pepper plants were set rather late in the spring. Their growth was further retarded by dry weather, and the first fruits were not set until the latter part of June. About this time a rainy period set in and lasted until the middle of July. During this period some rain fell almost daily, and both plants and fruits developed rapidly. On July 22 the entire field was examined carefully and not a single defective fruit was found. The earliest fruits were well matured at this time and subsequently ripened normally, but the younger fruits nearly all developed blossom-end spots during the ensuing drought.

EXPERIMENTAL RESULTS

In early summer of 1923, four plats, each six feet square, were set with 12 Pimiento pepper plants per plat. All were well watered and kept growing rapidly until August 20, when the first fruits were about an inch long. Water was then withheld from two of the plats. On August 30 two pods in these plats had developed spots at the blossom end. Between this date and September 24, ten more fruits in the dry plats developed spots, while none in the watered plats showed disease. Four of these spots were killed for histological study. The other eight were removed aseptically and placed on agar plates. All remained sterile.

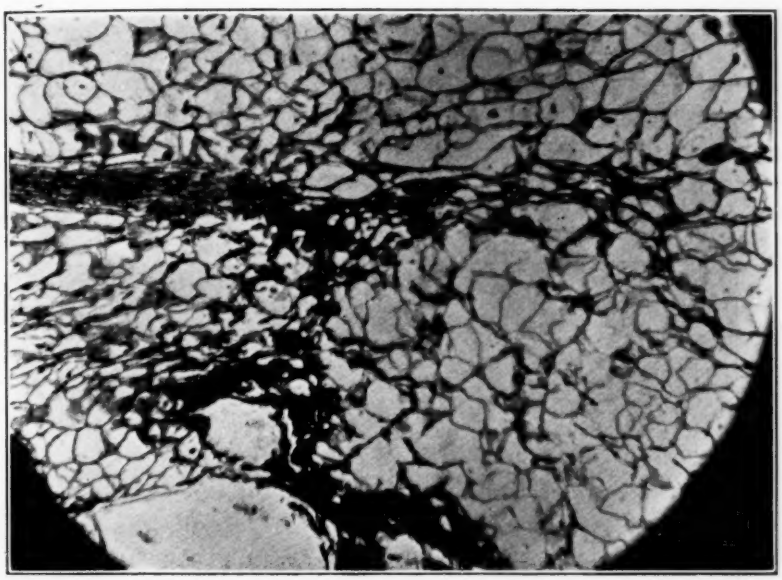

FIG. 2. Section of blossom-end rot spot, showing collapsed cells about end of vascular strand. Photomicrograph. ×65.

During the period of the experiment considerable rain fell. The air was moist and moisture seeped into the beds from the outside. It was therefore decided to run a series in pots so that the moisture could be better controlled.

On June 1, 1924, 26 plants of Pimiento pepper were set in 8-inch pots, and a similar number of Bell pepper plants were set in 12-inch pots. All were set on a greenhouse bench and were given plenty of water until fruits were about an inch long. Both the Pimiento and Bell series were then divided into two lots. Ten plants of each series were watered daily. The other 16 plants of each series received water at irregular intervals. Some

were watered only when wilting was severe, and others received a small amount of water each evening. The latter system proved slightly better than the former, since it kept the plants fairly vigorous but allowed slight wilting in the middle of every hot, dry day.

During extremely hot, dry days of August, plants of the well-watered pots showed slight wilting, and a few fruits developed blossom-end spots. This might be expected of plants with such a limited root system as potted plants in saturated soil are likely to develop. On the Bell plants receiving limited water supply, 31 fruits, or approximately 25 per cent, developed blossom-end spots; while only 3, or about 2 per cent, of the fruits on well-watered plants were affected. Pimientoes affected were approximately 10 and 3 per cent, respectively.

HISTOLOGY OF THE SPOTS

The spots for histological study were removed and killed within less than six hours after they became visible on the surface. Most satisfactory results were obtained with material killed in Schaffner's chrom-acetic acid, or in a mixture of equal parts of 95 per cent alcohol and glacial acetic acid, and

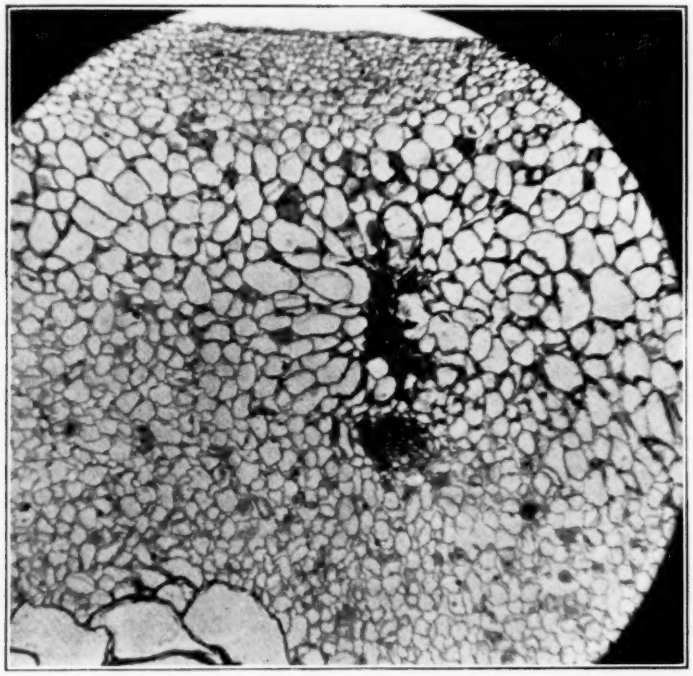

FIG. 3. Cross-section of spot, early stage, showing collapsed large cells above vascular strand. Photomicrograph. ×45.

stained with Haidenhain's haematoxylin. The flesh of the half-grown Pimiento pepper fruit is composed of a very thin cuticle, five to eight compact layers of comparatively small cells, about an equal number of layers of larger cells in which the small veinlets are embedded, and the single layer of very large cells lining the seed cavity. In the blossom end of the fruit there are numerous veinlet endings.

All the cells appear to be formed in the very young fruit, and the later enlargement of the fruit is due to the expansion of these cells. By the time the fruits are one and one-half to two inches long, certain cells lining the seed cavity have expanded until visible to the unaided eye as bladder-like projections over the inner surface, often a millimeter or more in diameter. The veinlets are surrounded by large, thin-walled cells.

As the fruit matures, the cell walls of the outer five to eight layers become cutinized; but this cutinization does not begin until the fruit is nearly full size, in vigorous plants when two to two and one-half inches in length.

It is near the end of the stage of rapid cell expansion, but previous to heavy cutinization, that the blossom-end spots are formed. The protoplasmic layer in the large cells surrounding the veinlet endings is very thin, and doubtless has very little power of recovery when once plasmolized. In the formation of the spots, these are the first to collapse (Figs. 2 and 3). Very often the tissue splits in this region and continues to collapse on the outer and inner surface layers (Fig. 4). In other cases collapse of the inner tissue is rapid and it does not split off from the outer layers.

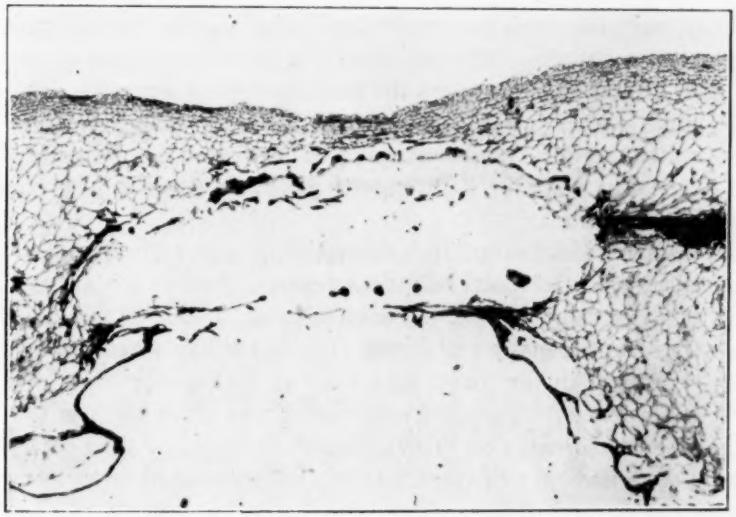

FIG. 4. Section of older spot, showing total collapse of inner tissue. Photomicrograph. ×25.

DISCUSSION

Field observations and experimental evidence have shown that two conditions are necessary for the production of blossom-end rot: (1) rapid succulent growth of the pepper fruits; (2) drought conditions must follow at once before the fruits become heavily cutinized.

The rot or spotting is always most severe on young plants. Under our field conditions a large percentage of the first-formed fruits are damaged, while those set later in the season are not severely damaged, unless there are extreme variations of wet and dry weather.

There are at least two reasons for the greater frequency of spotting of fruits on young plants. First, only a small number of fruits (one to five) are on the plant at this time and they grow larger and more rapidly than those on older plants which may support from fifteen to twenty-five growing fruits. The other is the poorly developed, shallow root system of the young plants which is incapable of obtaining sufficient moisture when the surface soil becomes dry, while older plants may be able to sustain growth from subsoil moisture.

Brooks (1) noted that blossom-end rot occurred on tomato plants growing in a nearly saturated soil, and from this he drew the conclusion that some factor other than drought might cause the rot. It may also be noted that on very hot, dry days spots developed on a few fruits on potted plants in wet soil; but I think the explanation lies in the poor development of young roots in a nearly saturated soil.

The vascular system of a pepper plant is more than sufficient to conduct an abundance of water to the leaves and fruits. Stems have been cut away until they were too weak to support their own weight, yet the remaining vascular tissue supplied sufficient water to prevent wilting of leaves or spotting of fruits. The roots are the limiting organs for water supply.

According to my observations on peppers, blossom-end spots never develop unless there is a slight drooping or wilting of the plant for at least a part of the day, and all cases of spotting may be explained by consideration of water relations.

The exact explanation of spot formation is not a simple matter however, because water relations between various organs of a plant are so imperfectly understood. Brooks (1) assumed that, in case of tomato blossom-end rot, water was actually withdrawn from the fruits, whenever a shortage of water occurred in the plant, by means of the greater concentration of solutes in the leaves. A similar assumption was made by Mix (4) in explaining the development of "drouth spot" in apples. Chandler (2) has shown by experimental evidence that such withdrawal of water from fleshy fruits for the support of leaves does occur in a large number of plants; and

this has been confirmed for pepper by my own experiments. Leafy branches removed from the plant wilt and dry more rapidly when the fruits are removed than when the fruits remain attached to the branch, and fruits attached to a severed leafy branch wilt more rapidly than those removed from the branch. It seems, therefore, that this sudden withdrawal of water by the wilting leaves is the primary cause of the spots. The negative pressure developed in the vascular system of the fruit causes withdrawal of water first from the cells surrounding the veinlet endings. These cells, poor in solutes and in protoplasmic content, have very little power of recovery after plasmolysis. This is especially true for young fruits in which the cells have reached nearly their maximum expansion but as yet contain little stored reserve food. The heavy cutinization of the outer cell layers of older fruits will usually prevent the collapse of this tissue, even when the interior cells become plasmolized.

SUMMARY AND CONCLUSIONS

1. A physiological spotting of the blossom end of pepper fruits is very common in the field and is the indirect cause of heavy loss to pepper growers.

2. The disease develops almost exclusively on half-grown, green fruits.

3. It is caused by drought following a period of rapid growth of the pepper plants and fruits.

4. The disease has been produced experimentally by withholding water from plants when the fruits were half-grown.

5. The spots are initiated by the collapse of the large, thin-walled cells surrounding the ends of vascular strands.

6. The spots usually become infected and enlarged by microorganisms.

7. Because of the small size of the pepper plant and the ease with which it can be grown under controlled conditions, the small size of the spots, and the thinness of the tissues involved, it is admirably suited for the study of drought spots.

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A ROT OF APPLES CAUSED BY BOTRYOSPHAERIA RIBIS

E. ALINE FENNER

Considerable literature has been published in the past regarding *Physalospora malorum* (Peck) Shear (*Sphaeropsis malorum* Peck) as a cause of black rot of the apple. Yet no one has made a systematic study of this disease of apples collected over a wide range of territory in the United States, nor has anyone done any intensive work in testing the pathogenicity of the organism causing it. The evidence given in this paper endeavors to establish the fact that in certain parts of the United States another fungus quite different from *P. malorum*, though related to it, is responsible for a rot very similar to the true black rot of the apple.

It was while engaged in some studies of black rot, in association with Dr. J. S. Cooley, the results of which will be published shortly, that this new rot was found. The causal fungus of this rot is *Botryosphaeria ribis* G. and D. (2). Morphological and cultural studies as well as inoculations have fully established the identity of the fungus from the apple rot tissue with that already known on apple and currant twigs. In view of the striking resemblance between the two fungi, not only as rots on apples, but also in culture, it is quite possible that some investigators have unwittingly been dealing with *Botryosphaeria ribis* instead of *Physalospora*.

In pursuing the investigations just referred to, apples were collected in 1923 from Mt. Alto, Pa.; Rockville, Md.; Middletown and Roanoke, Va.; Cornelia, Ga.; Bentonville, Ark.; Salem, Ind.; and Guelph, Ontario. Fungi were also isolated from rotting quinces and Kieffer pears. From all macroscopic appearances the fruit was infected with *Physalospora malorum*, some apples showing abundant young fruiting bodies and the concentric zones so characteristic of black rot. Even the sub-cultures of the fungi made from the decayed pulp, when grown on cornmeal agar, were so similar that no differences were noticed. It was only after the imperfect stages of the fungi fruited and a microscopic examination of the spores was made that the presence of the two distinct fungi was established.

The fungi were induced to fruit by following the methods employed by Shear, Stevens, and Wilcox (4). Transfers were made from the original sub-cultures to cornmeal paste in 100 cc. Erlenmeyer flasks. These flasks were kept in a greenhouse at Arlington Farm, Va., at a temperature ranging from 50°–70° F., with a much warmer temperature at midday. After a period of four to six weeks the cultures were carefully examined. Practically all of the ninety-seven cultures had fruited and a microscopic study revealed that ten of them, more than ten per cent of the total number, were

quite different from the *Physalospora malorum* which was expected. The spores of these fungi were hyaline and narrower than those of the pycnidial form of *P. malorum*, measuring 15–26 × 5–6 μ with an average of 20 × 6 μ. Careful study and comparison convinced the writer that they were the Dothiorella stage of *Botryosphaeria ribis*. Three of the apples infected with *B. ribis* came from Cornelia, Ga., one from Bentonville, Ark., and four from Arlington Farm, Va. The other two were made from Kieffer pears from Arlington Farm. That this fungus actually was *Botryosphaeria ribis* was strikingly confirmed by the fact that one of the cultures proved to be the pathogenic *Botryosphaeria ribis chromogena*, the cause of currant cane blight.

This particular culture isolated from an apple collected at the U. S. Experiment Farm at Arlington, Va., showed the deep pink color characteristic of the currant cane blight organism, when grown on a very starchy medium (2). To determine whether this chromogenic fungus was identical with the currant cane blight fungus, some inoculation experiments were made by Dr. Neil E. Stevens at Toms River, N. J. In the spring of 1924 two dozen currant plants of the "Wilder" variety were secured from a nursery in northern New York and planted in a location well removed from other currants. Early in June, when the plants had reached a stage in their growth which seemed most favorable for infection (2), one or more canes on each of eighteen were inoculated with mycelium from a pure culture of the chromogenic form from the apple. In all, twenty-six apparently sound currant canes were inoculated, six plants being kept for controls. The methods used were the same as those employed by Stevens and Jenkins (5). A small incision was made in the bark with a flamed scalpel and mycelium of the fungus inserted in the wound. Moist cotton was then bound around the cut. The control plants were wounded and tied up in a similar manner but without the inoculum. On August 10, after a period of two months had elapsed, twenty-two of the inoculations, or more than three-fourths of those made, had taken effect. The appearance of the dead canes was identical with that of canes killed by the currant cane blight fungus, and from the pith and wood of many of them isolation cultures were subsequently made. These cultures were compared with those of *Botryosphaeria ribis* and, so far as the writer could observe, were identical with them. The control plants and all canes not inoculated remained in a healthy condition.

That these fungi do actually cause a rot of apples was proved by inoculations. In the winter of 1923 and 1924 Grimes Golden, Ben Davis, and Jonathan apples were inoculated with mycelium from the ten cultures of *B. ribis* along with those of *Physalospora malorum*. The fruit was care-

fully selected in order to obtain uniformity of size and ripeness, care being exercised to discard any apples bearing stem punctures or other abrasions. After a thorough washing and rinsing in fifty per cent alcohol followed by sterile water, the apples were placed in damp chambers containing wet paper to maintain the proper moisture conditions. Inoculations were made by puncturing the skin with a sterile needle and introducing some of the mycelium. The inoculated fruit was kept at room temperature about five or six days and at the end of this period the rots were inspected. The apples were cut transverse to the core along a plane running midway between the calyx and stem-end. As in the case of the original apples from which the isolations were first made, the *B. ribis* rot was not distinguishable from the black rot caused by *P. malorum*, neither in respect to the decayed fleshy tissues of the apple nor in the general appearance of the skin. Even the measurements of the two rots varied through much the same range.[1] In fact, many efforts were made to find some conspicuous differences between the two rots on apples whereby one might distinguish the one from the other. Slight differences were observed but the rots caused by each fungus, however, showed such variations in respect to taste, firmness and appearance that repeated examinations of diseased fruits failed to reveal any definite means of discrimination.

In the autumn of 1924, fifty-eight more isolations were made from apples collected in Durham, N. H.; Snow Hill, Md.; Winchester and Covesville, Va.; Ft. Valley, Ga.; Bentonville, Ark., and Salem, Ind. In the study of these cultures the same method of procedure was followed that was used in the preceding year. When cultured on cornmeal in flasks, all the fungi fruited and among the number three chromogenic forms were found, one isolated from an apple collected at Covesville, Va., another collected at Ft. Valley, Ga., and a third at Arlington Farm, Va. Of the remaining fifty-five cultures six proved to be nonchromogenic *Botryosphaeria ribis*, two coming from Arlington, three from Ft. Valley, Ga., and the sixth from Winchester, Va. In all, over fifteen per cent of the cultures isolated from spots which were apparently black rot, proved to be the currant cane blight.

One of the most interesting phases of the second year's work was the appearance of the purplish pink color on certain apples artificially infected with the chromogenic form from Arlington Farm. This condition was not observed in the inoculations the first year, probably because the apples used in 1924 were decidedly green compared to the ones inoculated in the autumn of 1923 and therefore more starch was present in the tissues of the fruits. The results, then, in the two successive years were

[1] Graphs showing the measurements of the rots will appear in a joint publication with Dr. J. S. Cooley.

as might be expected, since it has already been noted that chromogenesis is in evidence only when the fungus is cultured on a very starchy medium. The results of this season's work not only confirm the data from last year's experiments, but the discovery of chromogenic forms from Covesville, Va., and Ft. Valley, Ga., shows that the fungus is not confined to any one region. It is quite possible in fact that *Botryosphaeria ribis* is rather generally present as a rot of apples in many parts of the United States.

That *Botryosphaeria ribis* occurs on cut apple limbs was announced about one year ago by Shear, Stevens, and Wilcox (4), but this fungus has never been reported in the United States as a rot of apples. Putterill (3), however, described a fungus in 1919 which caused a canker of apple trees in South Africa and which he also showed by artificial infection would cause a rot of apples. Although closely resembling *B. ribis* even to the chromogenic condition associated with the currant parasite, Putterill claimed the fruiting bodies of this fungus varied in size from those of the currant cane blight organism, and for that reason gave it the name *Botryosphaeria mali*. Shear, Stevens, and Wilcox (4) attach little significance to this difference as the stromata of the currant cane blight fungus are variable, their size depending to a great extent on the thickness of the bark on which they grew. The discovery that *Botryosphaeria ribis* causes a rot on apples in the United States strengthens the opinion already advanced (2) that Putterill's fungus and the one discussed here are one and the same organism.

Birmingham (1) recently reported a canker of apple trees in New South Wales caused by a fungus which he called *Dothiorella mali*, E. and E. His description of the fungus agrees with *Botryosphaeria ribis*. The writer has just received pure cultures of the apple fungus from Birmingham and has found that it develops on cornmeal flasks the chromogenesis characteristic of *Botryosphaeria ribis chromogena*, the currant cane blight fungus.

Birmingham does not mention the presence of this organism on the fruit of the apple but merely on the wood. However, the writer has recently inoculated apples with pure cultures of the fungus sent by Birmingham and has found that it causes a rot apparently identical with that caused by *Botryosphaeria ribis*.

SUMMARY

Evidence is presented in this paper to show that a fungus which causes a rot on the apple is identical with *Botryosphaeria ribis* and its form *B. ribis chromogena*.

The rot is very similar to that caused by *Physalospora malorum* and may easily be mistaken for true black rot.

When currant canes are inoculated with mycelium from the chromogenic form isolated from apples collected from widely separated regions, a disease

is produced apparently identical with that caused by *Botryosphaeria ribis chromogena*.

The identity of the fungus has been established by its morphological character, including spore measurements, and its pathogenic nature proved by several inoculation experiments both on apples and currant canes.

Apparently this fungus is identical with that reported by Putterill on apples in South Africa under the name of *Botryosphaeria mali*, and with that reported in New South Wales by Birmingham as *Dothiorella mali*.

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EFFICIENCY OF A SELF-MIXING DUSTER

RALPH E. SMITH

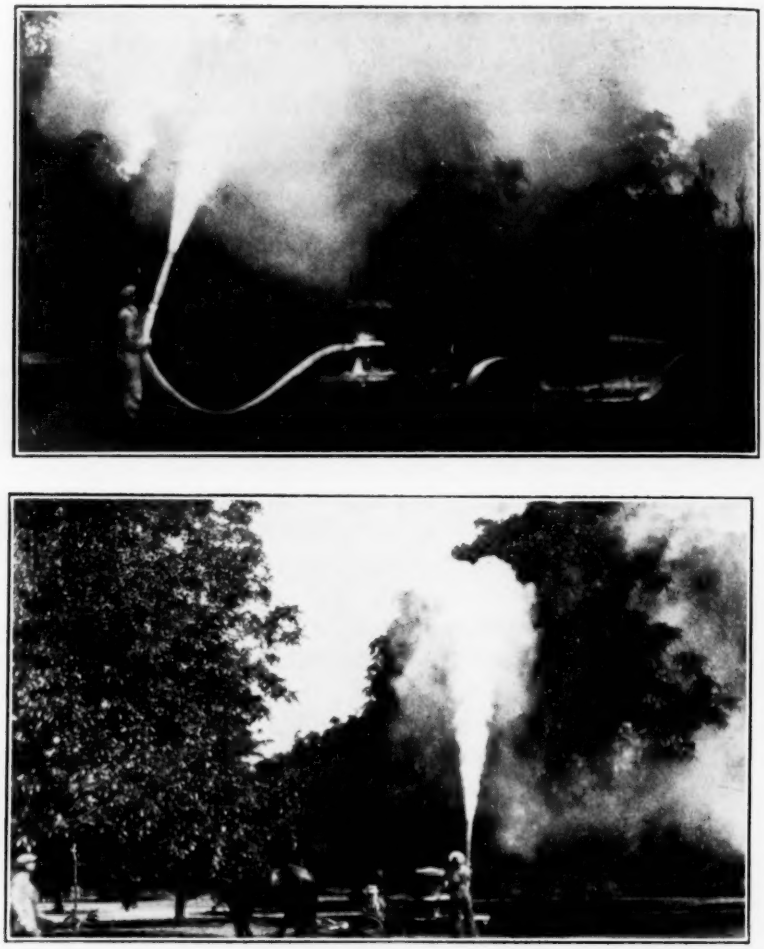
WITH ONE FIGURE IN THE TEXT

One of the obstacles to the success of the dusting method of applying fungicides and insecticides is the fact that mixtures containing different materials or proportions must be made up in the factory or by a separate preliminary mixing operation and cannot be varied or changed at will during the process of application. Furthermore, the extra cost of mixing, packing and transporting a large amount of low-grade filler like lime or sulphur adds a disproportionate amount to the price of the finished material by the time it reaches the farm. In the case of nicotine dust, a useful addition to many fungicidal materials, these considerations are particularly important. Nicotine is high priced and volatile and if the farmer could buy his own materials and mix the dust when and as needed, considerable economy could be effected together with the advantage of a freshly made mixture.

In a recent publication[1] we suggested the idea of a "self-mixing" duster —that is, a dusting machine having an agitator in the hopper so that dry pulverized materials like hydrated lime, sulphur, de-hydrated copper sulphate and commercial Bordeaux powder could be mixed together in any desired proportion in the machine itself. Nicotine dust is commonly prepared by mixing one of the commercial liquid preparations containing 40% nicotine as nicotine sulphate, with hydrated lime or sulphur, or a mixture of the two. In the proportions commonly employed the excess of dry material is sufficient to absorb the necessary amount of liquid and still remain in a dusty condition.

Objection has been made to the self-mixing idea as applied to nicotine dust on the ground that a sufficiently thorough mixture could not be made in this way. This depends, naturally, on the efficiency of the particular type of machine employed. That self-mixing can be applied in making nicotine dust was demonstrated by the following test, using a machine built in cooperation with this Division by the Bean Spray Pump Company of San Jose, California. Forty pounds of hydrated lime was dumped into the hopper of the duster and a two-pound can of "Hall's Nicotine Sulphate" (40% nicotine) sprinkled over it. The lid was then closed and the machine allowed to run for two minutes. At the end of this time a pint

[1] Smith, Ralph E., and J. P. Martin. A self-mixing dusting machine for applying dry insecticides and fungicides. Cal. Agr. Exp. Sta. Bul. 357: 497–506. 1923.



Dusting walnut trees with a self-mixing machine. Dust contains hydrated lime, dehydrated copper sulphate and nicotine.

glass fruit jar was filled with dust from the top of the mass (sample No. 1) and another from near the bottom (No. 2). The hopper was then closed and the machine run eight minutes longer. At the end of this time two more samples were taken (Nos. 3 and 4). The four jars were sealed and forwarded to the Division of Chemistry, California State Department of Agriculture, Sacramento, for determination of nicotine. The result was reported as follows: Sample No. 1, nicotine 1.89%; No. 2, 1.89%; No. 3, 1.88%, and No. 4, 1.87%. Theoretically, the mixture contained 5% of

the 40% nicotine solution, or 2% actual nicotine. A small amount of the solution probably stuck to the can in pouring into the hopper, but uniformity of mixing was the main consideration in this test rather than the actual amount of nicotine. The high degree of uniformity obtained throughout the whole mass of dust, even after the two-minute period, will be noted.

It is sometimes necessary to use a dust mixture containing considerably more nicotine than this. Another test was therefore made by using five and one-half pounds "Black Leaf 40" (40% nicotine, as sulphate) to 50 pounds of dust. This is 11% of the commercial solution, or 4.4% actual nicotine. A pint sample of the resulting mixture was sent to Sacramento for testing. The report showed the actual amount of nicotine in the sample to be exactly the same as the theoretical—namely, 4.4%. This represents about the limit of the use of the 40% solution for making nicotine dust, as the mixture tends to be moist and sticky.

These samples were taken from the hopper before the material had passed out into the discharge pipe. During the latter operation, in the type of machine developed by us, the dust passes through the fan. This breaks up any soft lumps and carries the mixing process still further. The demonstrated efficiency of machines representing the self-mixing idea and the great advantages, financial and otherwise, of such a device make the wider knowledge and application of this principle highly desirable. The cost of the dusting can be reduced and greater efficiency obtained by this method, particularly in the case of mixtures containing nicotine. The advantage of being able to vary the mixture at will at the time of application, just as different materials or amounts can be put into the tank of a liquid sprayer, is also an important one.

AGRICULTURAL EXPERIMENT STATION,
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A NEW FOOT ROT OF THE SWEET POTATO

J. J. TAUBENHAUS

WITH ONE FIGURE IN THE TEXT

This disease was first met with by the writer in 1914 and 1915 while he was connected with the Delaware Agricultural Experiment Station. The trouble was found at Seaford, Delaware, on tomato seedlings and sweet potato sprouts in cold frames. Cultures then made yielded a species of Macrosporium. However, owing to pressure of other work and change of positions, the writer was unable at that time to carry on further studies of this organism to determine its relationship to the foot-rot disease. During the last few years (1919 to 1921), while studying sweet potato and tomato disease at Troup and Jacksonville, Texas, a foot rot was found on sweet potato sprouts. The same trouble was also found on tomato seedlings in cold beds. In this connection it should be added that during 1920 Rosenbaum[1] described a foot rot of tomatoes from Delaware and New Jersey, which he attributed to the fungus, *Macrosporium solani* E. and M. In 1921, Pritchard and Porte[2] described a collar rot of tomato plants which they thought to be the same or similar to the one described by Rosenbaum, but which they believed to be caused by the fungus, *Verticillium lycopersici* Prit. and Port., although they also added that under certain conditions the same disease may be induced by *Macrosporium solani*.

The symptoms of foot rot both on sweet potato and tomato plants in Texas are characterized by deep, dark lesions at the foot of the plant (Fig. 1), which resemble the injury from Rhizoctonia. When these lesions become numerous, the affected host topples over and breaks. Numerous isolation cultures of the Texas material, both from sweet potato and tomato plants, frequently yield a pure growth of a Macrosporium fungus. It therefore became necessary to determine whether this disease was similar to the one described by Rosenbaum. Unfortunately, we were unable to secure cultures of his Macrosporium. In order to carry out inoculation experiments, sixteen 12-inch pots were filled with good garden soil and steamed for six hours at 15 pounds pressure. After cooling down, eight pots were planted with healthy, six-week-old tomato plants, and the re-

[1] Rosenbaum, Joseph. A stem disease of tomato caused by *Macrosporium solani* E. and M. (Abstract) Phytopath. **10**: 59. 1920.

[2] Pritchard, F. J., and W. S. Porte. Collar rot of tomato. Jour. Agr. Res. **21**: 179–184. 1921.

maining pots with healthy sweet potato sprouts. Six pots of each were used for inoculation purposes, and four as checks. The plants were permitted to grow undisturbed for several weeks. In this way both tomato and sweet potato plants made some new growth of stems and roots. The method of inoculation consisted in introducing into the soil an eight-day-old

pure culture of strains of the Macrosporium as they were isolated from affected sweet potato and tomato plants and grown on potato agar slants. The organism from the slant was first broken up in sterilized water, then poured around the foot of the plant and covered with sterilized soil. The

inoculated pots were kept moist by watering with sterilized water without, however, covering them with bell jars. The check plants were untouched, but were separated from the inoculated ones to avoid accidental infection. The results of these inoculations are given in table 1.

TABLE 1.—*Results of inoculating tomato and sweet potato plants with Macrosporium*

Source of organism	Host inoculated	Date inoculated	No. of pots inoculated	Result and date
Pure culture Macrosporium isolated from foot rot of tomato.	Tomato plants	May 10, 1922.	3	June 28, 1922. Typical foot rot lesions on 60 per cent of plants.
Check. No inoculation.	Tomato plants	Check. No inoculation.	2	June 28, 1922. All healthy.
Pure culture Macrosporium isolated from foot rot of tomato.	Sweet potato plants	May 10, 1922.	3	June 28, 1922. Typical foot rot lesions on 50 per cent of plants.
Pure culture of Macrosporium isolated from foot rot of sweet potato.	Sweet potato plants	do	3	June 28, 1922. Typical foot rot lesions on 50 per cent of plants.
Pure culture isolated from foot rot of sweet potato.	Tomato plants	do	3	June 28, 1922. Typical foot rot lesions on 30 per cent of plants.
Check. No inoculation.	Sweet potato plants	Check. No inoculation.	2	June 28, 1922. All healthy.

From this table it is seen that successful inoculations and typical foot-rot symptoms were obtained on the inoculated tomato and sweet potato plants when using a strain of Macrosporium fungus originally isolated from either of these two hosts. In this connection it should be added that reisolation cultures were made from the tissue of the artificially inoculated plants and the original fungus recovered.

Although no inoculations were made with the Macrosporium strain of Dr. Rosenbaum, it appears that the results shown in table 1 do not differ from those obtained by him. Further work is now in progress to determine whether the early blight of the Irish potato may be induced by using spores of *Macrosporium solani* from the foot rot of both sweet potato sprouts and tomato seedlings and vice versa.

TEXAS AGRICULTURAL EXPERIMENT STATION,
COLLEGE STATION, TEXAS.

A FIELD TEST OF MERCURIC CHLORIDE SOLUTIONS IN POTATO SEED TREATMENT

L. J. Cross

In the treatment of seed potatoes with corrosive sublimate solution, 1 to 1,000, for scab and Rhizoctonia, the strength of the sublimate solution decreases rapidly. To overcome this loss in concentration, some investigators advise the addition of one ounce of the sublimate to thirty gallons of the original solution after each treatment until it has been used five times, when it should be discarded. While this method tends to maintain the strength of the solution more nearly to the standard, yet the actual strength of the solution may be much higher or much lower than the standard, according to the condition of the potatoes used.

The withdrawal of the sublimate from the solution is not uniform with potatoes in different conditions. The more soil there is on the potatoes, the more rapidly will the sublimate be used up. The nature of the soil also has an influence upon the amount of sublimate withdrawn, some soils abstracting the sublimate more rapidly than others. Potatoes affected with decay or rot absorb the sublimate from solution very rapidly.

To overcome this objection, the following method of determining the strength of the sublimate solution has been devised. Solution required: Potassium iodide solution, five grams to the liter. Dissolve five grams of potassium iodide in distilled water and make to one liter.

Apparatus required:

1—25 cc. measuring cylinder.
1—100 cc. measuring cylinder.
1—1,000 cc. measuring flask.
1—10 cc. pipette.
1—300–500 cc. beaker, low type.
1—liter bottle for the potassium iodide solution.

Procedure: Measure out 25 cc. of the potassium iodide solution and place in a beaker. Fill the 100 cc. measuring cylinder to the 100 cc. mark with the sublimate solution to be tested. Titrate by pouring small quantities at a time of the sublimate solution from the measuring cylinder into the beaker and shaking until a permanent pink color or cloudiness is obtained. Note the amount of sublimate solution used. A test made of the solution as made up before treatment of the potatoes is started is used as a standard on the concentration of the other solutions after use in treating potatoes.

If the solutions have been properly made, it should take approximately 50 cc. of the 1 to 1,000 (4 oz. to 30 gals.) mercuric chloride solution to give the end-point against 25 cc. of the potassium iodide solution. This standard may be determined by dissolving one gram of pure mercuric chloride in one liter of water and titrating 25 cc. of the potassium iodide solution with a portion as has been described.

The sublimate solution used in treating the potatoes soon becomes dirty, and in the test a decidedly milky appearance of the solution is the end-point of the titration. This end-point has been checked with the potassium cyanide method for the determination of mercury and gives concordant results.

After long use of the sublimate solution, it becomes more difficult to determine the end-point of the titration when 25 cc. of the potassium iodide solution is used. Then one may use instead 10 cc. of the potassium iodide solution and titrate from a 25 cc. cylinder of the sublimate solution. Titrate to a decidedly milky appearance. The standard then will call for approximately 20 cc. of the sublimate solution. The exact standard will, of course, be obtained by titrating with the known mercuric chloride solution mentioned heretofore. With these small quantities it is possible to test the sublimate solution in the treating vat after a 1,200 bushel run.

This test has been used for four years under the direction of the author and 90 to 100 per cent killing of Rhizoctonia has been obtained in the treatment of about 50,000 bushels of seed potatoes.

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PHYTOPATHOLOGICAL NOTES

A New Bacterial Disease of Alfalfa.—In September, 1924, Prof. L. F. Graber called the writer's attention to a field of alfalfa near Freeport, Ill., which had been dying in spots in an unaccountable manner during the summer. A small spot which appeared to winterkill in a six-year-old field had grown larger after each cutting by the failure of plants around it to recuperate, until it had become about an acre in area, extending into an adjoining year-old planting. New smaller spots of dying plants were appearing in both the older and younger portions of the field. Examination of the tap roots of those plants which were putting out weak dwarfed foliage after the second cutting showed a brown discoloration under the bark which in advanced cases would slip readily from the root, leaving a woody core, yellow on the outside, but commonly of the usual white color at the center. Microscopic examination of these discolored roots revealed that the color was associated with the presence of masses of bacteria in a large number of the outermost vessels of the wood. In young plants apparently recently infected only a few vessels were found filled with bacteria having little color in mass. Later the color of the bacterial mass was darker, and finally the vessels were filled with a yellow substance in which the individual bacteria were not seen. Vessels thus filled with bacteria were found not only throughout the length of the larger roots, but in some of the stems with reduced foliage as high as six inches above the ground.

A survey of the older alfalfa fields around Monroe, Wis., discovered four additional fields infested with this disease. In one case, seven acres of six-year-old Grimm had lost half of a fine stand during the summer, and very few living plants remained uninfected. Another similar stand of Grimm was hardly less completely destroyed. A few plants diseased in this manner were found in a field at Madison, Wis. It is a fact of possible significance that at least three of these diseased fields have grown alfalfa before the present stand was sown.

An attempt to isolate bacteria from plants in each of these fields has given cultures of what appears to be the same organism in each case, and inoculations have produced the disease in plants brought into the greenhouse. Meantime it is desired to learn the extent of this trouble, and specimens from suspected fields will be greatly appreciated. The discoloration of the wood of the tap root just under the bark appears to be a distinguished characteristic of this disease. Unless it shall appear later that unusual conditions have made possible an extraordinary spread of this

trouble in the infested fields found, this disease appears to be a serious menace to alfalfa culture in the region where it has been found.—FRED REUEL JONES, Madison, Wis.

The weather and peach leaf curl in eastern Kansas in 1924.—The unusual weather of the spring of 1924 in the vicinity of Lawrence, Kansas, and its effect on a disease of the Chicasa plum caused by *Exoascus mirabilis* Atkinson has been described elsewhere (Mix, Biological and cultural studies of *Exoascus mirabilis*. Phytopath. **15**: 1925). The purpose of this note is to record the effect of the weather of that year and locality on the occurrence of peach leaf curl.

The following is a description of these abnormal weather conditions. There were showers on the twenty-seventh and twenty-eighth of March, without however developing into a rain of several hours' duration, such as is believed to be necessary for infection of peach leaves by *Exoascus deformans* (Berk.) Fuckel. A period of fair weather followed, broken only by showers on April 25, until a prolonged rain began on April 28.

The peach buds were beginning to open on March 28, and many infections might have been expected had the rain of that date been of longer duration. During April careful search was made in several unsprayed orchards in which peach leaf curl is usually abundant. On April 15 four curled leaves were found on one tree in one orchard. A short time later a few curled leaves were found on another tree in the same orchard. Except for these few specimens, no peach leaf curl could be found during that month. In a normal season the curl appears in this locality early in April.

On May 10 considerable curl was found. Most of this was on the one tree previously mentioned, but there was an occasional affected leaf on other trees. This late curl was different from the common type in that only small isolated areas of the leaf blade were involved. From one to several small lesions occurred on the same leaf. Apparently the leaf is more resistant to attack when partly grown, and the fungus is then unable to deform large areas of the blade.

The writer has observed this type of curl in other seasons and has believed it to be due to late infections, but evidence to prove this belief has been lacking. An experiment in artificial inoculation previously reported (Mix, Biological and cultural studies of *Exoascus deformans*. Phytopath. **14**: 217–233. 1924) shows that infection may occur after the leaves are expanded and partly grown.

The evidence here presented of two separate appearances of peach leaf curl, each following a possible infection period by about two weeks, seems to justify the following conclusions:

1. Infection of peach leaves by overwintering spores of *Exoascus deformans* may occur not only at the time of opening of the buds but also after the leaves are considerably expanded.

2. The peach leaf as it grows older is more capable of resisting the deforming effect of the fungus.

A further deduction from these observations is that overwintering mycelium of *Exoascus deformans*, if it exists at all, plays a negligible part in the incidence of the disease in the spring.—A. J. MIX, UNIVERSITY OF KANSAS, LAWRENCE, KANSAS.

An Easy Method of Mounting Superficial Fungi for Study.—In studying members of the Erysiphaceae, considerable difficulty has been found in quickly and easily obtaining mounts showing the vegetative parts of the fungus. When one desires to observe the relation of the conidiophores to the mycelium from which they arise and to study the catenulate nature of the conidia, it is necessary to have a slide showing the interwoven masses of mycelium in as normal a condition as possible. The method that has finally been evolved consists of using glycerine jelly slides which are easily prepared and used in the following way.

A camel's hair brush is dipped into melted glycerine jelly and a thin coat is spread over a part of a warm slide which is then allowed to cool. The leaf containing the mildew to be studied is pressed firmly with the fingers against the glycerine jelly slide. When the leaf is pulled away from the slide, much of the mycelium, conidiophores, conidia, and any perithecia present adheres to the surface of the glycerine jelly.

A mount made in this way may be observed under low power without a cover glass; or, by adding a few drops of water and then a cover glass, both low and high power may be used. Such a mount, after drying, is more than temporary, as it can be kept for some time; and its permanency may be increased by sealing with gold size.

This method is well adapted for teaching. The students are provided with glycerine jelly slides previously prepared. Their own mounts are made in the manner described with little difficulty. By this method the appendages of the perithecia remain intact and the mode of branching is easily seen. Likewise, the student sees the superficial nature of the mycelium, its tangled condition, and the catenulate manner in which the conidia are borne. For class study of the vegetative parts, *i.e.*, mycelium and conidiophores, clover mildew is better than that found on the lilac because of the more prominent conidiophores with from two to six conidia often remaining attached.

While this method has been developed for the study of powdery mildews, its use may be extended to other superficial fungi or those like the "downy

mildews'' in which the conidiophores are exposed. Very satisfactory mounts for study have been made of sooty molds, Trichopelts, Mycrothyriums, and Meliolas. It probably can be used satisfactorily for others of the Hyphomycetes besides the genus Oidium.—HOWARD C. ABBOTT, DEPARTMENT OF BOTANY, UNIVERSITY OF ILLINOIS.

Apple Blotch in New York State.—Apple blotch, caused by *Phyllosticta solitaria* E. and E., was found in April, 1924, in two planting of apple seedlings on a farm at Williamson, N. Y. In July, 1924, Mr. W. O. Gloyer, of the Geneva Experiment Station, reported (letter) the finding of blotch by Mr. Barney Blanch, state nursery inspector, in a nursery at Geneva, N. Y., from which one of the above lots of seedlings was obtained. These seem to constitute the first findings of blotch in New York State. Dr. M. B. Waite, of the U. S. Department of Agriculture, believed (letter to Dr. E. F. Guba, February, 1920) that he found the same fungus on foliage of apple in western New York about 1906, and again in 1910, but later decided that the fungus of those collections was *Coniothyrium pirinum*.

One planting at Williamson was very heavily infected. Eighty-six out of 100 trees were infected and it was estimated that 40 to 50 per cent of these seedlings were heavily cankered on the first year's growth. The spread to the 1923 twigs, however, was relatively slight. Budding on this stock in 1923 was almost a total failure. The relation of the blotch disease to this failure is of course uncertain. Only one plant was found in which the fungus had spread to the scion. On May 16, 1924, a number of trees from this planting were replanted on the University farm at Ithaca, N. Y., in a continuous row along with seedlings which did not show the disease. Five one-year-old trees of standard varieties were interplanted with the diseased trees at intervals of only a few inches. On September 26, eight of the original diseased trees were alive, and two of these showed a slight spread of the canker to the base of the current season's growth. No cankers could be found on the adjacent budded trees or on the seedlings which were clean at the time of planting.

All of the diseased lots of trees mentioned above were purchased from nurseries in Iowa. The practice of purchasing seedlings from the middle west seems to be increasing among our nurserymen. This obviously offers a constant source for importation of the blotch fungus into New York State and one that has undoubtedly existed for a number of years. However, coupled with the above observations, this situation lends support to the view that apple blotch is not likely to become a serious menace under our conditions. Nevertheless, it will be necessary to accumulate a greater body of facts before this or any other view may be considered as established. In

spite of the doubt that this pathogene will thrive in this state, the wholesale shipment of heavily-blotched, seedling nursery stock into territory hitherto free from it does not seem to be justified.—H. E. THOMAS, CORNELL UNIVERSITY, ITHACA, N. Y.

Cercospora Leaf Spot of Lettuce.—In Phytopath. **13**: 289, 1923, C. G. Welles describes as new a Cercospora disease of lettuce. He names the pathogene *Cercospora lactucae* and states, "I have searched through all available literature and I fail to find reference to such a Cercospora disease on lettuce." Stevenson (Jour. Dept. Agr. Porto Rico **1**: 105, 1917), described a Cercospora lettuce disease from Porto Rico in 1917. He also proposed the name *Cercospora lactucae,* which fact invalidates *Cercospora lactucae* Welles. The descriptions correspond so closely that they are probably of the same organism. Stevenson's description is much the fuller of the two. The disease, as described by Stevenson, has been called to the attention of American workers by Taubenhaus in his well-known book on vegetable diseases. (Diseases of truck crops, p. 145. New York, 1918.)—FRANK P. MCWHORTER, VIRGINIA TRUCK EXPERIMENT STATION, NORFOLK, VIRGINIA.